Microfossil assemblages as proxies to reconstruct anthropogenic induced eutrophication of two marginal Eastern Mediterranean Basins

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Abstract

Within the last decades the awareness about the socio-economic effects of pollution in general, and eutrophication in particular has increased and there is a rising demand for studies that provide accurate information about recent and past environmental changes that have taken place in the affected areas. A way to obtain this information is by establishing detailed palaeo-environmental reconstructions from time intervals prior and during the eutrophication events. The study of organic-walled dinoflagellate cyst (dinocyst) assemblages preserved in marine sediments has proved to be useful for reconstructing past changes in oceanographic conditions, primary productivity, and eutrophication level in eutrophicated areas. On the other hand, benthic foraminifera traditionally have been used for stratigraphic control and as paleoecological indicators. Over the last four to five decades, benthic foraminifera have been increasingly used as environmental bio-indicators, especially in polluted environments where their sensitivity to pollutants may be expressed by a modification of the assemblages. However, to do this, precise information about the relationship between present day upper ocean environmental conditions and these microfossil associations in modern surface sediment samples has to be established. For optimal applicability, these microfossil assemblages have to be tuned to local conditions and small scale features. The present study is based on analysis of organic-walled dinoflagellate cyst and benthic foraminifera from the Mediterranean Sea with special emphasis on the Abu-Qir Bay (Alexandria-Egypt) and Po-river (Italy) covering different levels of eutrophication patterns as well as regional anthropogenic influences.

The first two manuscripts of this thesis focus on the tuning of sedimentary assemblages to local environmental conditions. By using multivariate ordination techniques we investigated what environmental parameters have the largest determining roles influencing the species distribution in the study regions. The gathered information is used in the third study.

The first manuscript documents a study on the relationship between dinoflagellate cyst distribution in the marine surface sediments and the present-day upper water conditions based on a palynological analysis carried out on 34 surface sediment

samples taken along the Mediterranean Sea. The quantitative analysis of dinoflagellate cyst associations in surface sediments from the Mediterranean Sea revealed two cyst associations characteristic for: (1) offshore eastern Mediterranean regions and (2) areas located in the western Mediterranean Sea, the Strait of Sicily/NW Ionian Sea and/or the distal ends of the Po/Nile/Rhône River plumes regions. The offshore eastern Mediterranean stations are characterized by oligotrophic, warm, saline surface water, and high oxygen bottom water concentrations. The association is dominated by *Impagidinium* species, *Nematosphaeropsis labyrinthus, Pyxidinopsis reticulata* and *Operculodinium israelianum.* Temperature is positively related to the cyst accumulation of *O. israelianum*, but does not form a causal factor influencing the distribution of the other species in this group. The accumulation rates of species of the genus *Impagidinium* and *N. labyrinthus* increase with increasing nitrate concentrations in upper waters. The most characteristic species of group two are *Selenopemphix nephroides, Echinidinium* spp., *Selenopemphix quanta, Quinquecuspis concreta, Brigantedinium* spp., and *Lingulodinium machaerophorum*. At sites where these species are characteristically found, surface waters are characterized by high primary productivity associated with low SST and SSS, whereas bottom water oxygen concentrations are relatively low. Relative abundances and accumulation rates of *Selenopemphix nephroides* are positively related to upper water chlorophyll-*a* and nitrate concentrations indicating that this species might form a valuable eutrophication indicator in the study region. *Lingulodinium machaerophorum* is found in high abundances in samples located in river discharge plumes especially in the Nile plume. It might therefore form a suitable marker to trace past variations in river discharge, notably from the Nile.

The second manuscript focuses on the potential use of benthic foraminifera as pollution indicators. For this we selected one heavily polluted area from my home country as a case study. Eighteen sediment samples were collected from the Abu-Qir Bay, on the coast of Alexandria (Egypt) in the Eastern Mediterranean Sea. We compared the abundance and distribution of benthic foraminifera with results of chemical and sedimentological analyses. Statistical analysis (Cluster analysis and Canonical Correspondances Analysis) of the data shows two sectors with a distinct degree of pollution; an off shore less polluted sector and an onshore heavily polluted

sector. The first sector is dominated mainly by pollution sensitive species such as *Quinqueloculina vulgaris*, *Elphidium* spp., *Asterigerinata mamilla*, *Rosalina macropora, Ammonia beccarii, Triloculina trigonula, Peneroplis pertusus*, and *Quinqueloculina* spp. The onshore polluted areas are dominated by the pollution tolerant species *Ammonia tepida, Quinqueloculina lata,* and *Porosononion* spp., and in future can be used as bio-indicator of pollution. The results show that the reduced foraminiferal diversity, increase in dominance, and the frequent presence of deformed tests are linked with the heavy metal concentration, and therefore may be used as pollution indicators. These observations are of major importance for future research on the pollution history of the bay which is planned to be carried out in Alexandria University in near future.

In the third manuscript the information gathered in the first study is used to obtain insight into the eutrophication history of the last 80 years of the Po-river discharge plume by establishing a very high resolution (annual) palynological and geochemical record. The composition of dinoflagellate cyst assemblages and changes in their concentrations allows the identification of several eutrophication phases. A change to more eutrophic conditions of the regions can be observed already in the lowest part of the core deposited between 1932 and 1955. A strong change towards eutrophic condition is found between 1955 – 1970 caused by a combination of enhanced river discharge in the Adriatic Surface Water (ASW) source areas and anthropogenic activities. From 1980 onward, notably after 1990, water quality improves slightly probably as the result of reduced phosphate concentrations in the upper water masses.

In the appendix results from a pilot study carried out in the Abu-Qir Bay are documented. Here the phytoplankton abundance and community composition (with focus on dinoflagellates) are compared to the chemical and physical data of the waters at sampling points. This study reports several indications of water deterioration in the Abu-Qir Bay such as: a massive phytoplankton standing crop, low species diversity, frequent occurrence of toxic dinoflagellate harmful species, and a concurrent pattern between the phytoplankton and zooplankton cycles. The blooming of dinoflagellates (mainly *Gymnodinium* spp.) was observed in summer, while diatoms are dominant in winter. *Gymnodinium* spp. favours warm, saline,

stratified conditions in summer. Heavy metal pollution seems to have a negative effect on the dinoflagellate production, which is reflected by reduced concentrations in the polluted station compared to the other studied sites. *Prorocentrum* species, mainly *P. micans* and *P. triestinum*, can be used as indicator for eutrophication and pollution, whereas *Ceratium* spp. can be considered as pollution sensitive species.

The results of this thesis show that the Mediterranean Sea is a unique region with respect to the dinoflagellates cysts and benthic foraminifera communities. Detailed knowledge about the environmental preferences of the single species in modern oceans is the key for understanding changes in cyst assemblages and abundance during human induced eutrophication, and allows detailed reconstructions of various environmental and anthropogenic changes. This thesis enlarges the knowledge about both, spatial and temporal variations of different eutrophication proxies. Dinocysts and benthic foraminifera accurately reflect oceanographic and anthropogenic influences which forms the basis for their application for future eutrophication studies in aquatic ecosystems.

Zusammenfassung

In den letzten Jahrzehnten ist das Bewusstsein über die sozioökonomischen Effekte der Verunreinigung und Eutrophierung (Überdüngung) gestiegen und es herrscht eine steigender Bedarf an Studien, die Informationen über derzeitige und frühere Umwelt Veränderungen liefern, welche in betroffenen Gebieten stattfinden. Eine Möglichkeit, um an solche Informationen zu gelangen, ist die Erstellung einer detaillierten Paläo-Umwelt Rekonstruktion eines Zeitinterwals vor und während eines Eutrophierungsereignisses. Studien über organisch-wandige Dinoflagellatenzysten (Dinozysten) Vergesellschaftungen, präserviert in marinen Sedimenten, sind nützlich für die Rekonstruktion vergangener Veränderungen in den ozeanographischen Konditionen, der Primärproduktion und des Eutrophierungsstandes in überdüngten Gebieten. Andererseits wurden benthische Foraminiferen zur stratigraphischen Kontrolle sowie als palökologische Indikatoren genutzt. Seit 4-5 Jahrzehnten wurden im steigenden Maße benthische Foraminiferen als Umwelt Bio-Indikatoren genutzt, speziell in verunreinigten Gebieten, in denen die Sensibilität der Foraminiferen gegenüber Verunreinigungen zu Veränderungen der Artenzusammensetzung führen kann. Um dieses durchzuführen müssen präzise Informationen über den Zusammenhang zwischen heutigen Oberflächenwasser Umweltkonditionen und mikrofossilen Vergesellschaftungen in heutigen Oberflächensedimenten erzeugt werden. Für eine optimale Anwendbarkeit müssen diese Mikrofossilien auf lokale Konditionen und kleinskalige Kenndaten abgestimmt werden. Diese Studie basiert auf der Analyse von organisch-wandigen Dinoflagellatenzysten und benthischen Foraminiferen aus dem Mittelmeer mit speziellem Focus auf die Bucht von Abu-Qir (Alexandria-Ägypten) und dem Fluss Po (Italien), die verschiedene Ebenen in Eutrophierungsmustern sowie regionalen anthropogenen Einflüssen abdecken.

Die ersten beiden Manuskripte dokumentieren den Zusammenhang zwischen der Verteilung von Dinoflagellatenzysten in marinen Oberflächensedimenten und heutigen Oberflächenwasserkonditionen basierend auf einer palynologischen Analyse an 34 Oberflächensedimenten, welche entlang des Mittelmeeres genommen wurden. Die quantitative Analyse der Dinoflagellatenzystenvergesellschaftungen in

Oberflächensedimenten vom Mittelmeer zeigten zwei Zystenvergesellschaftungen, die charakteristisch sind für: (1) Der Mittelmeerküste vor gelagerte Regionen und (2) Gebiete im westlichen Mittelmeer, die Straße von Sizilien/NW Ionisches Meer und/oder das Ende der Abwasserfahne der Flüsse Po/Nil/Rhone. Die, der Küsten vor gelagerten Stationen des östlichen Mittelmeeres, sind gekennzeichnet durch oligotrophisch, warmes, salzreiches Oberflächenwasser und Sauerstoff reichem Bodenwasser. Die Artenvergesellschaftung wird dominiert durch Arten der Spezies *Impagidinium*, *Nematosphaeropsis labyrinthus, Pyxidinopsis reticulata* und *Operculodinium israelianum.* Temperaturen zeigen einen positiven Zusammenhang mit der Zysten Anreicherung von *O. israelianum*, aber keinen kausalen Zusammenhang zur Verteilung der anderen Arten in der Gruppe. Die Akkumulationsraten von *Impagidinium* und *N. labyrinthus* steigen mit steigender Nitratkonzentration im Oberflächenwasser. Die charakteristischsten Arten der zweiten Gruppe sind *Selenopemphix nephroides, Echinidinium* spp., *Selenopemphix quanta, Quinquecuspis concreta, Brigantedinium* spp. und *Lingulodinium machaerophorum*. An den Orten, wo diese Arten gefunden wurden, sind die Oberflächenwasser gekennzeichnet durch hohe Primärproduktion in Zusammenhang mit niedrigen Oberflächenwassertemperaturen und Oberflächenwassersalinitäten. Die Sauerstoffkonzentration im Bodenwasser hingegen ist dabei relativ gering. Relative Mengen und Akkumulationsraten von *Selenopemphix nephroides* stehen in einem positiven Zusammenhang zu Oberflächenwasser Chlorophyll a - und Nitratkonzentrationen, welche darauf hinweisen, dass diese Art einen wertvollen Indikator für Eutrophierung darstellen kann. *Lingulodinium machaerophorum* wird in hohen Mengen in Proben gefunden, die sich an den Enden der Abwasserfahnen der Flüsse, insbesondere des Nil befinden. Diese Art könnte deshalb einen brauchbaren Anzeiger darstellen, um frühere Veränderungen im Flusseintrag zu bestimmen, insbesondere am Nil.

Das zweite Manuskript befasst sich mit dem Gebrauch von benthischen Foraminiferen als Anzeiger für Verunreinigungen. Dafür wählten wir ein hoch verschmutztes Gebiet aus meinem Heimatland als Fallstudie. Achtzehn Sedimentproben wurden im östlichen Mittelmeer in der Bucht von Abu-Qir and der Küste Alexandrias (Ägypten) genommen. Wir verglichen die Häufigkeiten und die Verteilung von benthischen Foraminiferen mit den Ergebnissen von chemischen und

sedimentologischen Analysen. Statistische Analysen (Cluster Analysen und Canonical Correspondances Analyse) der Daten zeigten zwei Sektoren mit einem unterschiedlichen Grad an Verschmutzung; ein, den Küsten vor gelagerter, weniger verschmutzter Sektor und ein stark verschmutzter Sektor im Küstenvorland. Der erste Sektor wird dominiert von Arten, die sensibel auf Verunreinigung reagieren, wie *Quinqueloculina vulgaris*, *Elphidium* spp., *Asterigerinata mamilla*, *Rosalina macropora, Ammonia beccarii, Triloculina trigonula, Peneroplis pertusus* und *Quinqueloculina* spp. Die Gebiete im Küstenvorland werden dominiert von verschmutzungstoleranten Arten *Ammonia tepida, Quinqueloculina lata,* und *Porosononion* spp., welche als Bio-Indikator für Verunreinigung verwendet werden können. Die Ergebnisse zeigen, dass die reduzierte Foraminiferendiversität, der Anstieg in Dominanz und das häufige Auftreten von verformten Foraminiferen einen Zusammenhang mit der Schwermetallkonzentration darstellen und daraufhin als Verschmutzungs-Indikatoren genutzt werden können. Diese Beobachtungen sind von größter Bedeutung für künftige Forschungen der Verschmutzungsgeschichte der Bucht, welche in naher Zukunft an der Universität von Alexandria ausgeführt werden sollen.

Im dritten Manuskript werden die Informationen aus der ersten Studie genutzt, um einen Einblick in die Eutrophierungsgeschichte des Eintrages der Abwasserfahnes des Flusses Po der letzten 80 Jahre zu bekommen, an Hand der Herstellung einer hoch auflösenden (jährlichen), palynologischen und geochemischen Aufzeichnung. Die Zusammensetzung der Dinoflagellatenzystenvergesellschaften und Änderungen in ihrer Konzentration erlauben die Identifikation verschiedner Eutrophierungsphasen. Eine Änderung zu vermehrt eutrophen Konditionen der Regionen kann bereits im untersten Teil des Kernes, abgelagert zwischen 1932 und 1955, gefunden werden. Ein deutlicher Wechsel zu eutrophen Konditionen wurde zwischen 1955-1970 gefunden, verursacht durch eine Kombination aus vermehrtem Flusseintrag in den Adriatischen Oberflächenwasser Gebieten und anthropogenen Aktivitäten. Von 1980 an, besonders nach 1990, verbessert sich die Wasserqualität leicht, voraussichtlich durch reduzierte Phosphat Konzentrationen im Oberflächenwasser. Im Anhang befinden sich Ergebnisse einer Pilotstudie durchgeführt an Proben aus der Bucht von Abu-Quir. Dabei wurden die Phytoplankton Mengen und – Zusammensetzung, mit dem Fokus auf

Dinoflagellaten, verglichen mit chemischen und physikalischen Parametern des Wassers an den Probenpunkten. Diese Studie zeigt Anzeichen für eine Verschlechterung des Wassers in der Bucht von Abu-Quir, wie: einen Anstieg des Phytoplanktonbestandes, reduzierte Diversität, häufiges Auftreten von toxischen Dinoflagellatenarten und übereinstimmende Muster zwischen Phytoplankton- und Zooplankton Zyklen. Die Blüte von Dinoflagellaten (hauptsächlich *Gymnodinium* spp.) wurde im Sommer beobachtet, während Diatomeen im Winter dominieren. *Gymnodinium* spp. bevorzugt warme, salzreiche, stratifizierte Konditionen im Sommer. Schwermetall Verunreinigungen scheinen einen negativen Effekt auf die Dinoflagellatenproduktion zu haben, welche sich in reduzierten Konzentrationen in den verunreinigten Stationen wieder spiegelt im Gegensatz zu den anderen untersuchten Orten. *Prorocentrum* Arten, vorzugsweise *P. micans* und *P. triestinum*, können als Indikatoren für eutrophisch verunreinigte Gebiete genutzt werden, während *Ceratium* spp. als sensible Art bezeichnet werden kann.

Die Ergebnisse dieser Dissertation zeigen, dass das Mittelmeer eine einzigartige Region im Hinblick auf Dinoflagellatenzysten und benthischen Foraminiferen darstellt. Detailliertes Wissen über das bevorzugte Lebensumfeld einzelner Arten im heutigen Meer ist der Schlüssel für das Verständnis von Veränderungen in Zystenvergesellschaftungen und Zystenhäufigkeiten während menschlich verursachter Eutrophierung und erlaubt eine genaue Rekonstruktion von verschiedenen Umwelt Veränderungen. Diese Dissertation vergrößert das Wissen über die räumlichen und zeitlichen Variationen der unterschiedlichen Eutrophierungs Proxies. Dinozysten und Foraminiferen reflektieren äußerst genau ozeanographische und anthropogene Einflüsse, welche die Basis bilden für ihre Anwendbarkeit und weitere Eutrophierungsstudien in aquatischen Ökosystemen.

Chapter 1

Introduction

1.1 Preface, scientific context, and main objectives

Anthropogenic induced pollution is one of the most important problems threatening the Mediterranean ecosystems. The modification and destruction of marine and coastal habitats through improper development practices and poor management are very significant problems. Anthropogenic stress on the Mediterranean marine environment can be categorised according to the following scheme:

Land-based sources of pollution (sewage and urban run-off, urban solid wastes, persistent organic pollutants (POPs), heavy metals, organohalogen compounds, radioactive substances, nutrients, suspended solids, hazardous wastes).

Habitat destruction and physical alteration (shoreline construction and alteration, wetland and salt-marsh alteration, marine waters and coastal watershed alteration).

Off shore and marine-based pollution (petroleum hydrocarbons from shipping activities, marine litter).

Emerging issues (biological invasions, overexploitation of fisheries resources, expansion of aquaculture, increasing appearance of Harmful Algal Blooms (HABs) (EEA, 2006).

The population of the Mediterranean countries was about 450 million in 1996 and it is estimated to reach 520–570 million by 2030 (EEA, 1999). This constantly increasing population is one of the major pressures on the quality of the Mediterranean water.

Along the Mediterranean coastline, 131 pollution hot spots were recorded (UNEP/WHO, 2003). These hot spots are point pollution sources or polluted coastal areas which affect human health, ecosystems, biodiversity, sustainability, and economy. Of these hot spots, 26% are urban, 18% industrial and 56% mixed (urban

and industrial) (UNEP/MAP, 2003). Among these hotspots are Abu-Qir Bay and discharge plume area of the Po-river. Additionally, 59 sensitive areas (marine areas under threat of becoming pollution hot spots) have also been identified along the Mediterranean coastline. All these pressures have led to the degradation of environmental quality in the Mediterranean areas (EEA, 2006).

Eutrophication of coastal and adjacent offshore areas is a major problem in the Mediterranean Sea area. The most pronounced effects of the anthropogenic induced eutrophication e.g. due to overloading with nitrogen and phosphorus are known to be expressed as a reduction in biodiversity, increasing occurrence of algal (toxic) blooms and/or anoxic events that can lead to massive benthos and fish mortality as well might form a severe risk for human health (e.g. Spatharis et al., 2007). This can have strong economical effect e.g. as reduced income from fishery, mariculture and tourism.

The major problems in marine coastal environmental science revolve around the intersection of humans and the environment. Pollution problems are correlated with population density, which has driven significant efforts to monitor aquatic ecosystem in order to assess their status. These monitoring efforts are important because they allow scientists and managers to begin to relate drivers, stressors and effects. Establishing the relationships between drivers, stressors and effects provides managers with a sound scientific basis on which to build consensus on solutions to problems. Environmental remediation and restoration activities are often based on the assumption that removal of anthropogenic stressors will result in a return of the system to optimum ecological integrity. The ecological condition of a system is a function of physical, chemical, and biological processes, most of which are affected by anthropogenic and natural stressors. Without data from the analysis of biological and chemical indicators in sediment cores and an understanding of historical human activities, it is difficult to assess ecosystem integrity.

Chemical and biological methods are often used in assessing the effects of human influence on the ecology of aquatic ecosystem. However, using these methods to quantifying these effects and distinguishing them from natural ones is hampered by the lack of data prior to the onset of human influence. Such retrospective data are important for comparison between the biota prior to and during the influence of

human activity, to be able to quantify the effects. Secondly, they may serve as a reference to evaluate the extent of environmental recovery in response to subsequent measures to reduce the effects. Parts of the biota are preserved in the sedimentological record as fossils and have the potential to record the effects of human influence and climatic change on the environment, and therefore increase our understanding of cause-effect relationships. However, to do this precise information about the relationship between present day upper ocean environmental conditions and fossil associations in modern surface sediment samples has to be established. Both dinoflagellates and benthic foraminifera have proven useful as environmental indicators. Micropalaeontological studies based on sedimentary dinoflagellate cyst and benthic foraminifera assemblages are extremely useful to study such environmental change. The cyst forming dinoflagellate association in upper waters is well known to reflect in detail the environmental conditions which several species being characteristic for eutrophication (e.g. Dale and Fjellsa˚, 1994; Saetre et al., 1997; Thorsen and Dale, 1997; Dale et al., 1999; Dale, 2001; Pospelova et al., 2002; Shin et al., 2010). The benthic foraminiferal assemblage on the other hand, is strongly related to the rate and quality of organic matter deposition on the ocean floor as well as the redox state of the bottom sediment and the bottom boundary layer (Armynot du Châtelet et al., 2004; Debenay et al. 2006; Tsujimoto et al., 2008; Al-Zamel et al., 2009). However, the studies that investigate the relationship between the sedimentary dinoflagellate cyst and benthic foraminifera assemblage in relationship to environmental conditions in the water column have revealed that although there are generalities, every region has its own specific association and relationship. Consequently for an adequate reconstruction of past changes in environment a so called “local tuning” has to be performed for the research area.

The thesis has been carried out from October 2007 to October 2010 at the Department of Geology, in the historical geology/palaeontology group. The thesis is cumulative and consisted of three papers and an additional prototype of a manuscript in the appendix. The papers are preceded by a general introduction, and followed by conclusions and scientific perspectives. The introduction provides the scientific background of the thesis and builds the superstructure for the separate manuscripts. The four manuscripts are first-authorships and are either published, have been or will be submitted to peer-reviewed journals.

Here we study responses to nutrient enrichment in the Mediterranean Sea, with special focus on the Po-river system and the polluted Abu-Qir Bay studying organic dinocysts and benthic foraminifera to assess eutrophication effects. We first study the relationship of fossil remains of these organisms with modern environmental parameters and recent human influence, to identify their applicability as indicators of the effects of human activity such anthropogenic eutrophication in the Mediterranean coastal waters. Using this information we reconstruct the eutrophication history of the distal part of the Po-river discharge area as case study.

The aim of the first manuscript (*Chapter 2, Distribution patterns of recent organic-walled dinoflagellate cysts in relation to environmental parameters in the Mediterranean Sea*) is to establish an inventory of the recent organic-walled dinoflagellate cyst distribution in surface sediments from the Mediterranean Sea to provide an elaborated concept for the organic-walled dinoflagellates. A mapping of the present day distribution patterns of dinoflagellate cyst species in the Mediterranean Sea and comparing them to environmental variables has been carried out in order to reveal species-specific environmental preferences. In this study both of the relative and absolute abundance have been taken into consideration, to obtain a real picture of dinoflagellate cyst preference. The results presented in the first manuscript are of crucial importance to the rest of the thesis in general and to the third manuscript in particular. They show that the fossil dinocyst association in the Mediterranean Sea forms a very accurate tool to reconstruct, in detail, the environmental changes notably eutrophication in the aquatic environment in general and in river systems in particular.

The second manuscript *(Chapter 3, Spatio-temporal distribution of neritic benthic foraminifera in relation to anthropogenic activities in Abu-Qir Bay, Alexandria, Egypt)* focus on the question to which extent we can use modern benthic foraminifera as environmental and pollution indicators in one of the hot spot polluted area in the south eastern Mediterranean Sea and to explore which species or combination of species provides the best information about changes in pollution level and environmental conditions. The survey conducted is based on chemical and sedimentological analyses that are integrated with benthic foraminifera analyses.

The third manuscript *(Chapter 4, 80 years trophic history of the distal part of the Po-river discharge plume: Evidence from a high temporal resolution dinoflagellate cyst record*) presents detailed high resolution cyst analysis of a sediment core covering the past 80 years of deposition at the distal part of the Po-river discharge plume area. Comparison of the cyst record with the environmental information that is present for the last 30 years allowed a detailed reconstruction of both natural and anthropogenic induced eutrophication during the last 80 years.

The fourth manuscript *(Appendix, Eutrophication stress on the phytoplankton community of the Abu-Qir Bay, southeastern Mediterranean Sea, a dinoflagellate perspective)* presents one of the most commonly used methods for investigating eutrophication, the analysis of plankton communities, where changes in abundance and species composition associated with known eutrophication may indicate the extent of eutrophication. The seasonal patterns of the phytoplankton community (especially the dinoflagellates) are compared to different pollution, physico-chemical, and trophic conditions. A set of phytoplankton community eutrophication indicators are determined for the polluted eutrophicated Abu-Qir Bay (south-eastern Mediterranean Sea).

1.2 Areas of investigation

1.2.1 The modern Mediterranean Sea

1.2.1.1 Geographic location and climate

The Mediterranean Sea is a land-locked marginal sea of the Atlantic Ocean and it is divided by the Strait of Sicily into two main sub-basins; the western and eastern Mediterranean. It is bordered by Europe to the north, Asia to the east, and Africa to the south.

Climatologically, the Mediterranean resides at the transition between the high- to mid-latitude and the subtropical atmospheric systems, which interact and, in a way, may be regarded to compete with one another (Lionello et al., 2006). As a consequence, the Mediterranean climate depends on the latitudinal shift of these climate systems with the seasons, and typically varies between mild, wet winters and hot, dry summers (Eshel, 2002; Rohling et al., 2009).

Specifically, the winter regime is dominated by mild and wet conditions related to the southward movement of the temperate westerlies from central and northern Europe (Lolis et al., 2002), with occasional outbreaks of cold polar/continental air masses funnelled through the valleys of the northern Mediterranean margin (Maheras et al., 1999). In summer, warm and dry conditions prevail as a result of the high pressure belts of the subtropics drifting northwards in the Northern Hemisphere (during May to August), and southwards in the Southern Hemisphere (during November to February). During the winter, the high-pressure belts drift back towards the equator (Rodwell and Hoskins, 1996; Saaroni et al., 2003; Ziv et al., 2004). It is worth noting that before the anthropogenic curtailment of the freshwater discharge of the Nile River, another climate system, namely the northern African monsoon, used to have an impact on the Mediterranean system and specifically on the basin's hydrography. The influence of the African monsoon on the Mediterranean hydrography is however indirect as there is no actual latitudinal displacement of this climate system onto this region but rather a modulation of the basin's freshwater inputs via the Nile River and other North African drainage systems (Rohling and Bryden, 1992; Rohling et al., 2002, 2004; Scrivner et al., 2004; Skliris and Lascaratos, 2004).

1.2.1.1 Oceanography

The Mediterranean Sea is a concentration basin; evaporative losses (E) exceed the freshwater inputs resulting from precipitation (P) and river runoff (R) (Gilman and Garrett, 1994; Garrett, 1996). This feature is apparent over the Mediterranean as a whole as well as over the two sub-basins in particular, as exemplified by the two-layer exchange systems at Gibraltar (Bryden and Kinder, 1991) and at the Strait of Sicily (Garzoli and Maillard, 1979), in which the eastward surface flow of warmer, fresher surface waters overlies the westward flow of colder, saline (i.e., denser) subsurface waters (Astraldi et al., 1999).

The Mediterranean circulation is driven by water exchange through the various straits, wind stress, and thermohaline fluxes, with the latter depending on the basin's freshwater and heat budgets (Robinson et al., 2001). The vertical distribution of the Mediterranean water masses includes the surface (0-200 m), the intermediate (200-

600 m), and the deep waters (> 600 m) (Pinardi and Masetti, 2000; Tsimplis et al., 2006).

In the northern sectors of the basin the surface patters are dominated by cyclonic gyres. In the southern sectors surface waters derived from the Atlantic Ocean flow eastwards through currents, jets (western basin) and anticyclonic gyres (eastern basin) (Pinardi and Masetti, 2000) (Fig. 1a), while undergoing temperature gain and net evaporative loss, with the latter leading to strong west-east salinity gradients. In the northern Levantine basin the advection of winter (cool) northerly air masses neutralizes the temperature gain, thereby causing the formation of Levantine intermediate water (LIW) in the Rhodes Gyre (Buongiorno Nardelli and Salusti, 2000). This water mass subsequently settles between 200 and 600 m and spreads out in the entire Mediterranean basin to eventually enter the Atlantic Ocean at Gibraltar (Fig. 1b).

The interaction of the LIW with cold surface waters in the northern basins of the Mediterranean governs the deep-water formation processes, which is responsible for the deep-sea ventilation of the entire basin (Pinardi and Masetti, 2000). Sites of deep water overturning are the Gulf of Lions for the western basin (western Mediterranean Deep Waters, WMDW), and the Adriatic (Adriatic Deep Waters, ADW), and Aegean (Aegean Deep Waters, AeDW) Seas for the eastern basin (Fig. 1c). Overall, the Mediterranean thermohaline circulation has proven to be primarily tied to the basin's negative freshwater budget, whose variations are dominated by processes acting in the eastern Mediterranean (Rohling and Bryden, 1992).

As a result of its circulation pattern strong E-W directed environmental gradients exist in the upper water column of the Mediterranean Sea (e.g. POEM Group, 1992; Pinardi and Masetti, 2000). Temperature and salinity increase eastwards, as the surface water experiences warming and consequently evaporation during the transport. The water masses are nutrient depleted and generally oligotrophie to ultra-oligotrophie with decreasing tendency towards the east, with the exception of eutrophicated coastal areas

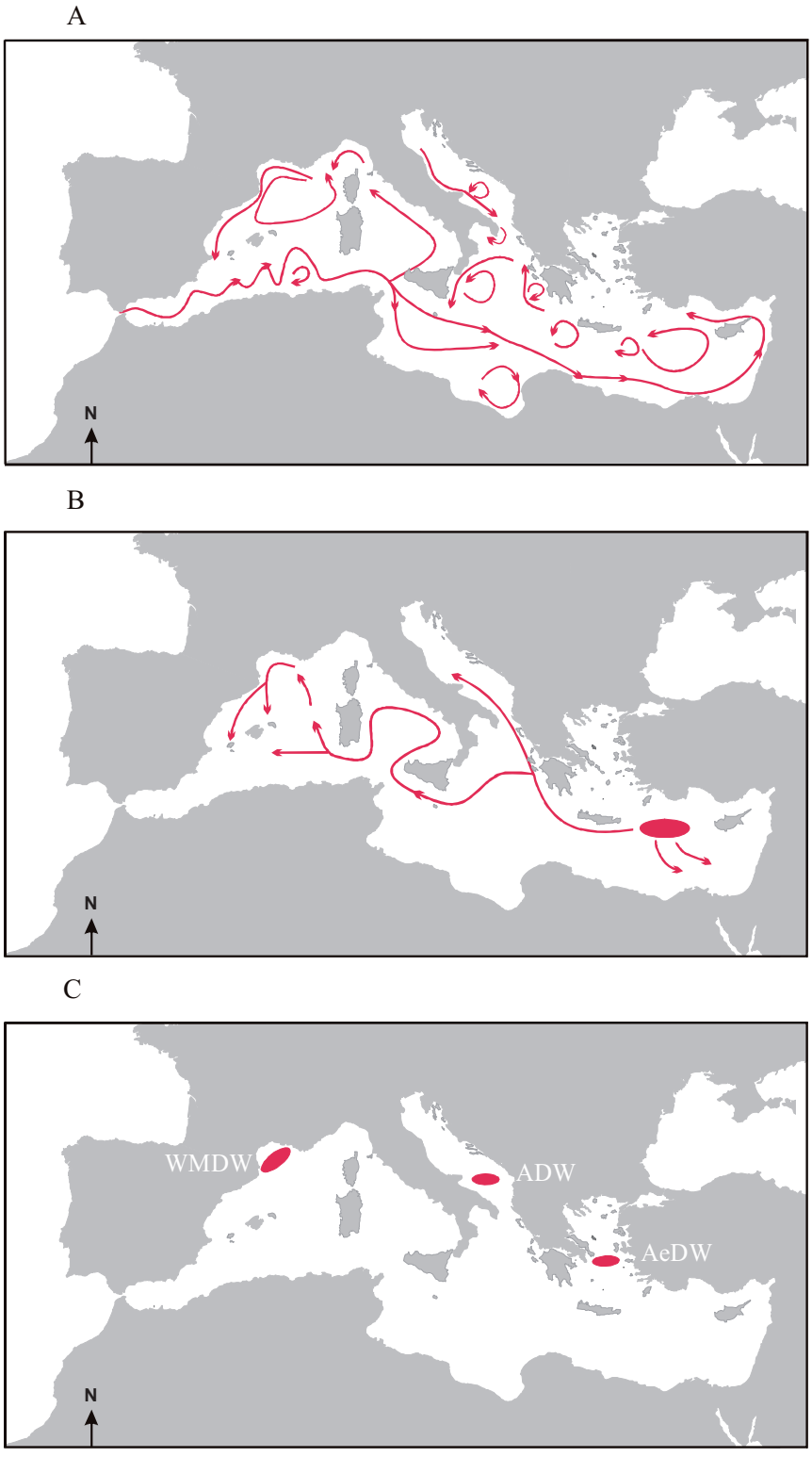

Figure 1. Water mass circulation in the Mediterranean Sea. (a) Schematic representation of the major features of the surface water circulation. (b) Circulation pattern of the Levantine Intermediate Waters (LIW). (c) Sites of deep water overturning (dense water formation) (modified after Pinardi and Masetti, 2000).

We zoomed into two selected eutrophicated areas of the eastern Mediterranean Sea; the Abu-Qir Bay which is characterized by high pollution level, and the Po-river system.

1.2.2 Abu-Qir Bay: Characteristic features

Abu-Qir Bay is a side basin of the Eastern Mediterranean Sea in the vicinity of the city of Alexandria (Egypt). The area has a strong economical value for the local Egyptian economy as it is the major source area of fisheries in the region (El-sayed and Moharram, 2007). This semicircular shallow basin is lying at 35 km to the east of Alexandria city between Abu Qir Peninsula (west) and the Rosetta branch of the Nile (east), with a shoreline about 50 km long. It lies between 30^{o} 4'- 30^{o} 21' East and 31^{o} 16'-31^{o} 30' North (Fig. 2). The bay is relatively shallow with a depth ranging from less than one meter along the coast, increasing gradually away from the shore to reach a maximum depth of about 15 m. The bay has a surface area of about 360 km^2, its mean depth is 12 m and the volume of water is about 4.32 km^3. The amount of freshwater discharged into the bay is estimated as 2130 x 10^6 m^3/y (Abdel-Moati, 2001).

Abu Qir Bay was considered to be one of the most profitable fishing areas before it was polluted by the disposal of sewage and industrial effluents. The environment of the Bay is characterized by a gradient from mixotrophic/oligotrophic conditions in the outer bay area that is connected to the open Eastern Mediterranean Sea, to extremely eutrophic conditions in the inner bay in the vicinity of the city of Alexandria. There are strong indications that the rate of pollution has increased remarkably during the last two decades related to the expansion of the Egyptian economy and of the city of Alexandria (EEA Report, 2006). It receives different types of pollutants. The Main sources of pollution are: Abu Qir Drain, Lake Edku, and Rosetta Branch of the River Nile in the eastern part of the bay (Sharaf EL-Din et al., 1980; Saad et al., 1980).

Figure 2. Study area of Abu-Qir Bay.

1.2.3 Po-river: Characteristic features

The second selected area of study is the Po-river system which is one of the major European river systems. The study area focuses on the gulf of Taranto where the distal end of the Po-river discharge plume can be traced.

It has been reported that the high-intensity algal blooms, leading at times to fish kills and unpleasant conditions for tourism, periodically occur along the Italian coast of the Adriatic Sea (Buljan and Zore-Armanda, 1976). It is thought that these are

mainly caused by the influx of waters from the Po (Innamorati and Giovanardi, 1992; Marchetti et al., 1995; Boldrin et al., 2002, 2005), which is Italy's most important river. This occurs especially when the river floods, but can also be due to particular climatic conditions or occur when wind and currents push the river's waters (which normally flow out into the sea and disperse there) towards the coast (Cacciamani et al., 1992). Logically, changes in amount and quality of the Po-river discharge have large effects on the marine environment (Penna et al., 2004). Pilot studies have documented the high potential of the natural archives in the region for palaeo-environmental and palaeo-climatic studies (Cini Castagnoli et al., 1999; Sangiorgi and Donders, 2004).

Presently, the climate in the Adriatic Sea region during winter is regularly perturbated by mid-latitude wind systems. Two major wind systems affect the Adriatic basin. During winter, the dominating wind is the Bora, a dry and cold north-eastern wind. The other is the Scirocco, a characteristic wind of the southern Adriatic, which brings rather humid and relatively warm air from the southeast into the region. The Bora, which produces appreciable buoyancy loss through evaporative and heat loss, induces both wind-driven and thermohaline circulation, and is thus important for deep-water formation in the Mediterranean Sea. (e.g. Artegiani et al., 1989; Bignami et al., 1990; Orlic et al., 1992).

Surface waters of the Adriatic Sea are characterised by an inflow of Mediterranean Surface Waters (MSW, from the Ionian Basin into the Adriatic. Furthermore an outflow of relatively fresh Po-river discharge water, which mixes with the inflowing MSW, can be observed along the Italian coast (Artegiani et al., 1989; Orlic et al., 1992). In comparison with MSW this water is enriched in nutrients and characterised by relatively high bioproduction (Jorissen, 1988). This modified Po-river discharge water (further on referred to Po discharge water) flows out through the Strait of Otranto and can be traced as far as Sicily. Apart from a surface inflow of MSW, a subsurface inflow of Levantine Intermediate Water (LIW) can be observed. In winter deep water is formed during cold, dry air outbreaks ("Bora" winds blowing from the Northeast) resulting in enhanced evaporation and cooling of the surface waters. A detailed study about the mechanisms, position of the source locations of the deep water and characteristics of the involved water masses, is given in Orlic et al. (1992).

The deep water formed in the Adriatic Sea flows out over the bottom of the Otranto Strait and forms Eastern Mediterranean Deep Water (EMDW).

1.3 Dinoflagellates

1.3.1 Dinoflagellates in general "biological background"

Dinoflagellates (Division Dinoflagellata (Bütschli, 1885) Fensome et al., 1993) are a eukaryotic, predominantly single-celled aquatic group of organisms. Dinoflagellates (subdivision Dinokaryota) have, apart from a few exceptions, a unique and characteristic type of cell nucleus, termed a dinokaryon, which distinguishes the Dinokaryota from all other eukaryotic organisms. Dinoflagellates typically occur as motile cells with two characteristic flagella, a transverse and a longitudinal flagellum which are in most cases ventrally inserted (= dinokont dinoflagellates). The transverse flagellum is ribbon-like running along the outer edge of the motile cell and is typically located in a transverse groove, called the cingulum (Fig. 3). The longitudinal flagellum projects posteriorly with its proximal end usually in a longitudinal furrow, termed the sulcus (Fig. 3). The synchronous movement of both flagella results in the eponymous spirally swimming behaviour (Greek δίνη = dino, meaning vortex, whirl). The cingulum generally divides the cell in an anterior (= epitheca or episome) and in a posterior part (= hypotheca or hyposome). The ventrally situated sulcus roughly divides the cell into right and left halves (Fig. 3). The motile cell is covered by a complex series of membranes termed the amphiesma which includes a layer of so-called amphiesmal vesicles. In many taxa, these vesicles contain cellulosic plates (= thecal plates) which vary interspecifically in thickness and grade of ornamentation. Species having cellulosic plates are called thecate, in contrast to athecate taxa whose vesicles lack thecal plates. The sum of all cellulosic plates which tightly fit together constitutes the theca and generates a distinct tabulation pattern.

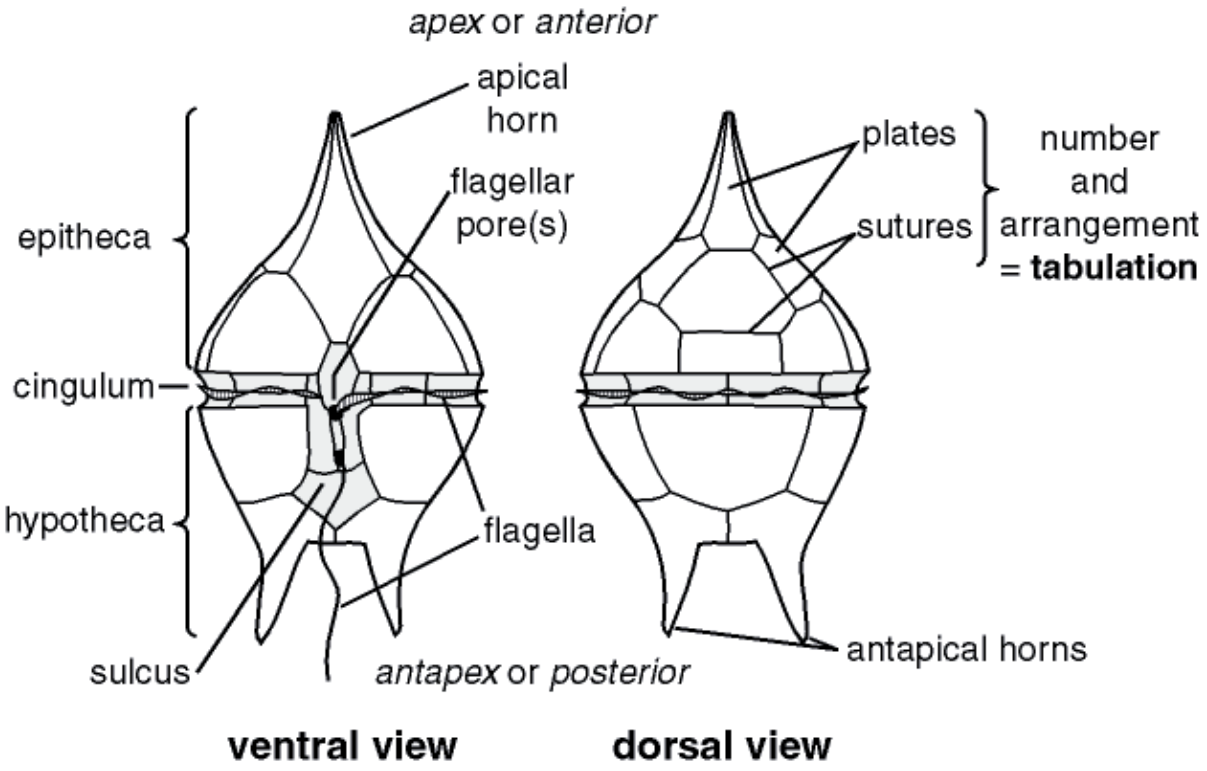

Figure 3. The principal morphologic features of a typical thecate motile cell of a dinokont dinoflagellate (modified after Evitt, 1985).

Nutritional strategies of living dinoflagellates are quite diverse, varying from phototrophic/photophototrophic (= photosynthetic), heterotrophic/phagotrophic (= ingestion of dissolved or solid organic matter), auxotrophic (autotrophs, unable to synthesize essential nutrients by themselves) to mixotrophic (= heterotrophs bearing chloroplasts). Some dinoflagellates even have more than one strategy (see Gaines and Elbrächter, 1987 for details). Most dinoflagellates live planktonic or benthic, but symbiotic (e.g., as 'zooxanthellae' in reef-building corals) or parasitic forms are also known.

Dinoflagellates inhabit most aquatic environments of all climatic regions, ranging from marine to freshwater habitats, and have also be found in snow and interstices of wet sand (Taylor, 1987). However, most of the taxa are marine and they reach their highest diversity in the shelf areas (Stover et al., 1996). Dinoflagellates constitute a major component of the marine phytoplankton and represent important primary producers in the oceans.

Some of the dinoflagellates are able to make the sea glow at night (bioluminescence). Dinoflagellates are simulated to bioluminescence by deformation of their cell membrane generated by shear forces induced by strong stirring of water, such as breaking waves or rapid swimming of fish or invertebrates. Although there are many uncertainties of the function of the bioluminescence, it has been shown that it can act as a sort of "burgular alarm system" (Abrahams and Townsend, 1993). The flashing serves to attract the predators of the dinoflagellate's grazer. The dinoflagellates render themselves dangerous as prey upon attack because they generate a signal identifying the location of food to individuals two trophic levels up the food chain.

1.3.2 Life cycle of dinoflagellates

The life cycles of living dinoflagellates taxa evinced to be extremely diverse and multi-faceted ranging from simple to highly complex. The general life cycle normally includes a simple asexual (=vegetative) reproduction of the haploid motile cell through mitosis, meaning the division of one cell into two daughter cells (binary fission). However, more intricate life cycles occur in many free living forms which are characterized by a non-motile phase during which the cell is enclosed in a cyst (Fig. 4). The cyst walls consist either of an organic polymer called dinosporin (organic-walled dinoflagellate cysts), calcite (calcareous dinoflagellate cysts), silica (siliceous dinoflagellate cysts) or, like the motile cells, of cellulose. With the exception of the latter, all forms are fossilizable since they are relative resistant to dissolution and/or microbial disintegration. Four major types of cysts are distinguished according to their function: 1) temporary cysts (motile cells that temporarily lose their flagella and outer wall as a reaction to stressful conditions), 2) vegetative cysts (metabolically and/or reproductively active, non-motile cells covered by a continuous wall), 3) digestion cyst, which is extremely rare in dinoflagellates and is being formed after digestion of food. Until now no forms with high preservation potential have been recorded, and 4) resting cysts, representing a dormant stage in which most life processes are highly reduced. They are generally formed as part of the sexual reproduction. Concerning the resting cysts, since they are the result of sexual fusion, they are diploid and thus zygotic resting cysts, termed hypnozygotes. A motile diploid zygotic cell (planozygote) forms after the fusion of haploid motile cells (gametes) and resembles the former haploid cells. Planozygotes

often show paired flagella and typically increase in size before they become non-motile hypnozygotes (= cysts). This process from planozygote to hypnozygote with the formation of membranes to serve as the future cyst wall and the final loss of the thecal plates is called encystment. Resting cysts are generally produced inside the dinoflagellate theca. The cyst shape may resemble that of the motile cell or may comprise a more or less spherical central body which can bear processes or crests. The obligate period of dormancy following the encystment is highly variable in length and may last from 12 hours to several months (Pfiester and Anderson, 1987). Following upon the dormancy period, the hypnozygote emerges through a distinct aperture called archeopyle (excystment) which resulted from the loss of an intraspecifically consistent number of plates (= operculum). The cycle closes with meiotic divisions producing new haploid cells and starting a new phase of vegetative reproduction (Fig. 4). Most fossil dinoflagellates are believed to represent hypnozygotes (Pfiester and Anderson, 1987; Fensome et al., 1996). However, since the sexual reproduction of dinoflagellates, which produces fossilizable resting cysts, is known for only 13-16% of all extant species (Head, 1996).

1.3.3 Encystment and Excystment

Numerous issues concerning the encystment of dinoflagellates remain to be unsolved. Especially the question which factors trigger sexuality and successive cyst formation is currently debated (e.g., Godhe et al., 2001; Olli and Anderson, 2002). In many culturing experiments it has been shown that sexuality and cyst formation can be induced by nutrient depletion of especially phosphate and nitrate (e.g., Anderson et al., 1984; Anderson and Lindquist, 1985; Blanco, 1995; Olli and Anderson, 2002). Nevertheless, in long-term field studies it has often been observed that maximum cyst formation occurs during, or just after periods of maximal vegetative cell division with nutrient concentrations not being depleted (e.g., Ishikawa and Taniguchi, 1996; Montresor et al., 1998). High encystment success of *Scrippsiella* cf. *lacrymosa* has even been reported to be related to high nutrient conditions (Olli and Anderson, 2002). It is suggested that a possible solution for this discrepancy could be that nutrient concentrations in the local water conditions surrounding the individual dinoflagellate specimens might be the crucial factor. Cells are thought to initiate sexuality as the intracellular pools of limiting nutrients reach critical levels due to a

change in uptake at low ambient nutrient concentrations or due to some other environmental stress. However, in the field the evidence on cyst formation in relation to nutrients is indirect and can often not be found (Kremp and Heiskanen, 1999; Godhe et al., 2001). Other factors that might influence encystment of cysts are temperature, day length, irradiance and an endogenous encystment rhythm (e.g., Anderson and Keafer, 1987; Pfiester and Anderson, 1987; Costas and Varela, 1989; Kremp and Heiskanen, 1999; Godhe et al., 2001). It has been shown that several dinoflagellates are able to produce cyst in a limited temperature window. Temperature itself however, is not a triggering factor. Recently, it has even been suggested that cell contact related to cell density might be crucial (Uchida, 2001). In general, encystment of dinoflagellates is not related to stress but is rather favoured by optimal conditions for vegetative growth.

Excystment can be triggered or inhibited by several factors such as temperature, light availability, oxygen concentration and endogenous rhythms. In culture conditions excystment can be often be inhibited by cold storage whereas temperature increase might induce germination (e.g., Dale, 1983; Binder, 1986; Pfiester and Anderson, 1987; Kremp and Anderson, 2000). Species have a species specific temperature window in which germination can take place. Light availability is generally thought as being essential to germination and it has been shown that a pulse as short as one second might be sufficient (e.g., Binder and Anderson, 1986; Nuzzo and Montresor, 1999). Hatching of cysts is observed in culture experiments where the cysts were stored in the dark (e.g., Kremp and Anderson, 2000). It is, however, hard to say if short light pulses of low radiance in these experiments were completely avoided. Anoxic conditions inhibit cyst germination in most, if not all, dinoflagellate species (Kremp and Anderson, 2000; Persson, 2001). Germination is reduced under low oxygen conditions.

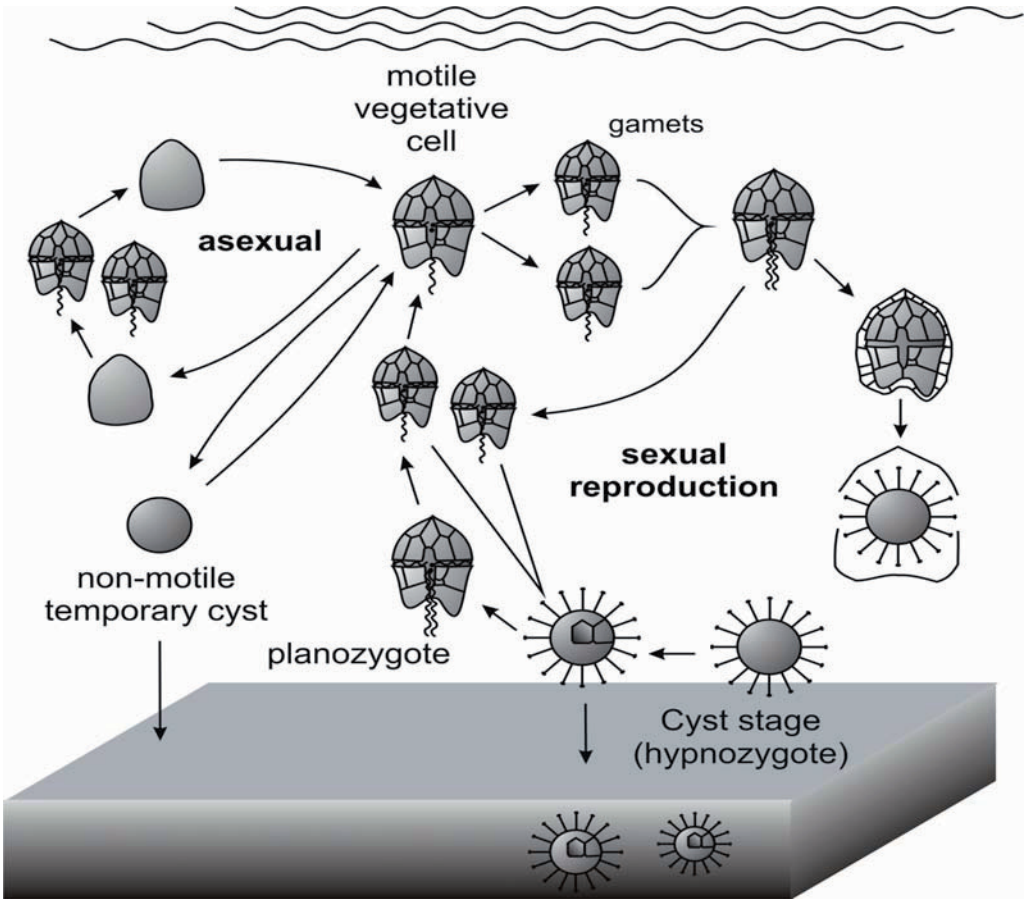

Figure 4. Simplified life cycle of cyst producing dinoflagellates (after Dale, 1986).

1.3.4 Dinoflagellate preservation

Previous studies on accumulation rates of cyst species indicate that post-depositional degradation occurs in all marine environments with oxygenated bottom waters. Table 1 shows the grouping of dinoflagellate cyst species with respect to their sensitivity to oxygen availability in pore waters according to Zonneveld et al (2001). Incubation experiments in natural environments show that this is a very fast process severely changing the dinoflagellate cyst association with a concentration decrease of about 30% of *Brigantedinium* cysts in 15 months of exposure to oxygenated waters whereas exposure to anoxic waters does not significantly alter the cyst association. This indicates that diagenetic effects can easily overprint other signals such as cyst production, environmental changes or post-depositional lateral transport of cysts e.g. as the result of downslope transport of sediments from poor oxygenated shelf areas to the well ventilated abyssal plains and deep ocean. This does not mean that the initial palaeo-environmental signal is lost in all cases; the extent to which the original signal

remains depends on the overprint. Consequently, P/G ratio and A/H ratio which are commonly used as proxies for estimating past productivity and eutrophication signal have to be used with extreme care. Species selective degradation has to be taken into account when interpreting such ratios. Since the majority of resistant cyst species are formed by phototrophic/mixotrophic gonyaulacoids whereas the majority of the most vulnerable cyst species are formed by heterotrophic dinoflagellates. Correcting for selective degradation can be carried out by concentrating on the palaeo-environmental signal of resistant species only, or by using a degradation index (Zonneveld et al., 2008).

Table 1. Dinoflagellate cyst species grouped with respect to their sensitivity to oxygen availability in pore waters according to Zonneveld et al. (2001).

Extremely sensitive	Cysts formed by Protoperidinium species
	(*Brigantedinium* spp.) and *Echinidinium* species
Moderately sensitive	*Lingulodinium machaerophorum*
	Protoceratium reticulatum
	Pyxidinopsis reticulatum
	Spiniferites species (including *Spiniferites bentorii, Spiniferites mirabilis, Spiniferites pachydermus* and *Spiniferites ramosus)*
Resistant	*Nematosphaeropsis labyrinthus*
	Impagidinium aculeatum
	Impagidinium paradoxum
	Impagidinium patulum
	Impagidinium plicatum
	Impagidinium sphaericum
	Operculodinium israelianum
	Pentapharsodinium daleii
	Polysphaeridium zoharyi

1.3.5 Dinoflagellates ecological interest

The relationship between modern assemblages in the sediment and the environmental conditions in the upper water column is the basis for the use of dinoflagellate cysts as a proxy for oceanographic conditions. Studies from quaternary open oceanic environments (e.g. Mudie and Short, 1985; de Vernal and Giroux, 1991; Edwards and Andrle, 1992; Matthiessen, 1995; Marret and de Vernal, 1997; Rochon et al., 1998; Marret and Zonneveld, 2003; Holzwarth et al., 2007; Pospelova et al., 2008;

Bouimetarhan et al., 2009; Holzwarth et al., 2010; Elshanawany et al., 2010) suggest that it is basically nutrient concentration, turbulence, sea surface temperature and sea surface salinity which are related to the composition of the dinoflagellate cyst assemblage. Concurrently, dinoflagellate cysts became increasingly used for paleoceanographic and paleoenvironmental studies to document aspects of changes in the ocean conditions during the period of major global climatic and environmental changes, especially in neritic highly-productive environments where the microbial degradation of the organic matter causes carbonate dissolution restricting the use of calcareous proxies (e.g., planktonic foraminifera, diatoms and coccolithophores. Furthermore, it has become apparent that also the organic-walled dinoflagellates are a useful tool for the reconstruction of paleoenvironmental conditions, not only for short-term variations during the Late Quaternary, but also for the long-term climatic variations of the Cenozoic era. Recent work suggests that cysts are potential indicators of human influence in aquatic ecosystem, both reflecting eutrophication (increased rate of supply of organic matter to an ecosystem as defined by Nixon (1995) and more industrial pollution (Dale and Fjellså, 1994; Dale, 1996; Sætre et al., 1997; Thorsen and Dale, 1997; Dale et al., 1999; Dale, 2001; Matsuoka ,1999, 2001; Pospelove et al., 2002; Chmura et al., 2004; Shin et al., 2010).

Another proposed effect of human influence of increased interest both to scientists and general public is the causes of unusual or harmful algal blooms. Several species are known to be able to produce toxins that can lead to massive fish kills and/or form a problem for human health as inbreathing of the toxins or consumption of infested sea-food can cause diarrethic and/or paralytic shellfish poisoning (e.g. Hallegraeff, 1993; Buskey, 2008). Although such blooms have often been linked to human induced eutrophication, detailed studies of some recent cases have not shown convincing cause-effect relationships. There is therefore a need for a better understanding of the dynamics of phytoplankton populations and of such blooms (GESAMP, 1991).

1.4 Benthic Foraminifera

1.4.1 Foraminifera: biological overview

Foraminifera are single-celled marine organisms. They live either benthic or planktonic. The soft tissue (protoplasm) is largely enclosed within a harder shell

(test). The test may be organic (not mineralized), agglutinated (constructed of foreign particles cemented together by the foraminifer), composed of calcium carbonate or, in rare cases, silica (Goldstein, 1999). Secreted calcium carbonate test foraminifera are again subdivided into three major groups, microgranular (e.g. Fusulinina), porcelaneous (e.g. Miliolina) and hyaline (e.g. Globigerinina) The shells are commonly divided into chambers which are added during growth, though the simplest forms are open tubes or hollow spheres. These chambers are mostly less than 1mm across. Each chamber is interconnected with the next by an opening (foramen) or many openings (foramina). This opening gives the animal-class the name. Foraminifera are characterized by the presence of granuloreticulose pseudopodia which are thread-like extensions of the ectoplasm often including grains or tiny particles of various materials (Lee and Anderson, 1991).

Concerning the trophic mechanisms, the Foraminifera utilize a broad range of feeding mechanisms and nutritional resources, including grazing, suspension feeding, deposit feeding, carnivory, parasitism, the direct uptake of DOC, and symbiosis (Goldstein, 1999).

1.4.2 Life Cycle of foraminifera

The Foraminifera are united by a life cycle characterized by a fundamental alternation of sexual and asexual generations that has become secondarily modified in some groups (Fig. 5) (Goldstein, 1999). An asexually produced haploid generation commonly form a large proloculus (initial chamber) and are therefore termed megalospheric. Sexually produced diploid generations tend to produce a smaller proloculus and are therefore termed microspheric. Importantly in terms of the fossil record, many foraminiferal tests are either partially dissolved or partially disintegrate during the reproductive process (Goldstein, 1999).

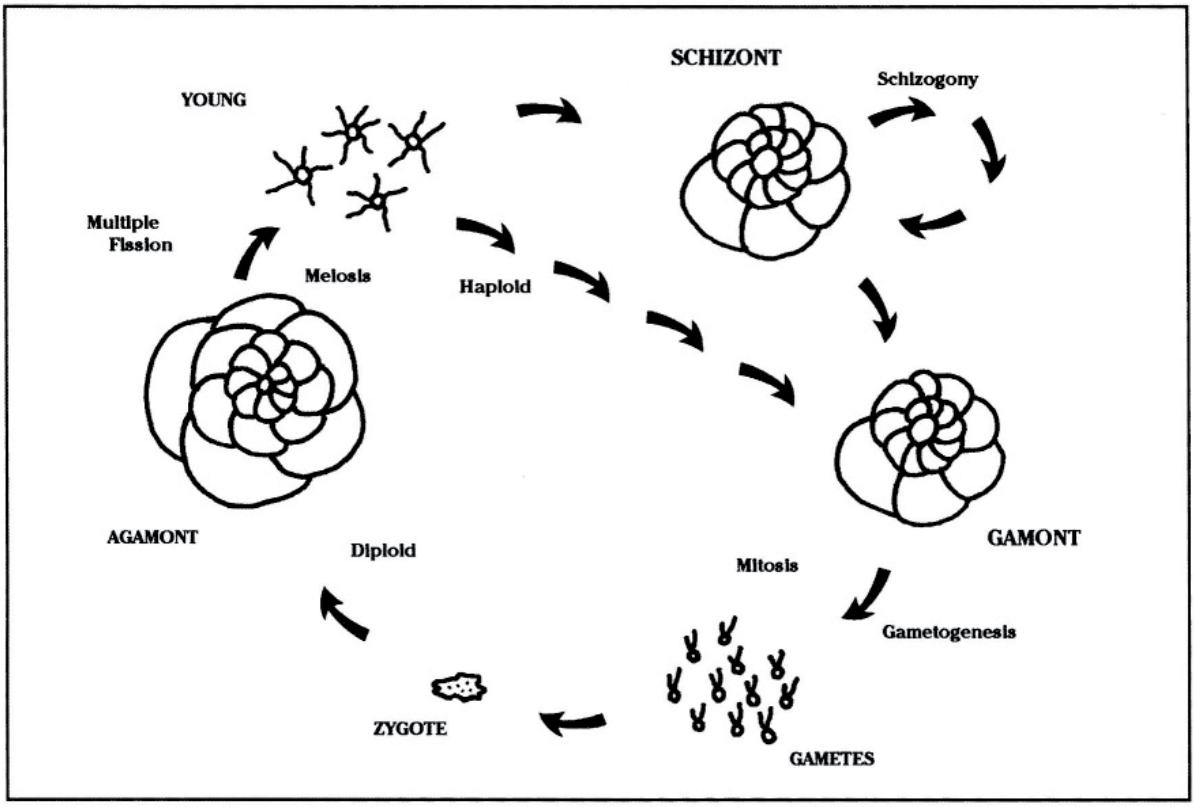

Figure 5. Outline of the foraminiferal life cycle which classically includes a regular alternation between a haploid, uninucleate, megalospheric gamont, and a diploid, multinucleate, microspheric agamont. Meiosis occurs in the agamont as part of multiple fission, and gametes are produced by mitosis. In some species, the life cycle also includes a schizont which is produced from the agamont and reproduces asexually. It can interject numerous successive asexual cycles. Meiosis typically occurs in the agamont. However, the type of nuclear divisions in the schizont has not been documented for any species (after Goldstein, 1999).

1.4.3 Importance of Foraminifera and ecological interest

Foraminifera are among the more abundant and most conspicuous protozoa in most marine and brackish water habitats. Their durable shells (tests) are an important component of marine and paretic sediments (Lee et al., 2002). Numerous researchers have used foraminiferal studies in environmental investigations. Foraminifera play a significant role in global geochemical cycles of inorganic and organic compounds. Their tremendous taxonomic diversity and cosmopolitan occurrence make them potential bioindicators for different environmental conditions. Their hard tests are readily preserved, and can record evidence of environmental stress over time. Many other factors favour their use as bioindicators: (1) they are ubiquitous to marine environments; (2) they live on and in sediment, which can act as a sink for pollution;

(3) foraminifers are relatively small and abundant, permitting statistically significant sample sizes to be collected quickly and relatively inexpensively, ideally as a component of comprehensive monitoring programs; (5) reef-building, zooxanthellate corals and foraminifers with algal symbionts have similar water-quality requirements; and (4) the relatively short life spans of foraminifers as compared with long-lived colonial corals facilitates differentiation between long-term water-quality decline and episodic stress events (Kramer and Botterweg, 1991).

Foraminifera are widely used in paleoceanography and paleoclimatology. There are two major ways in which fossil foraminifera can be used as proxies. The first is by enumerating abundances of different species in a fossil sample and to infer past habitats by the presence or absence of certain (key) species. Such reconstructions can be improved by increasing our knowledge about the habitat preferences of modern species. In order to investigate temporal and spatial distributions of living foraminifera against an environmental background, field studies are conducted in which foraminiferal distributions and environmental parameters are recorded. In many cases, the abundance of a species is found to be positively correlated to a range of values of an environmental variable. The abundance of that same species in a fossil sample is then used to reconstruct values for that environmental parameter in those samples. The second way in which foraminifera are used is by analyzing the chemical composition of their tests. Ratios of carbon and oxygen isotopes in foraminiferal calcite contain valuable information on, for instance, past oceanic temperature and global ice volume. Furthermore, during calcification by the foraminifer, trace elements (like Mg, Ba, Cd, Zn, Cu) can be incorporated in the $CaCO_3$-lattice by substituting Ca. Besides the concentration of trace elements in the seawater, the amount of a trace element that is incorporated in the carbonate is usually a function of several environmental parameters. In the case of magnesium, the incorporation into foraminiferal calcite is mainly determined by the temperature of the seawater. Hence, Mg concentrations in fossil calcite (commonly expressed as Mg/Ca ratios) reflect sea water temperatures at the moment when the calcite was produced. The dependency of trace element/Ca ratios on temperature, salinity, pH, as well as its dependency of cellular activity of the foraminifer is uncertain for most trace elements. Therefore they need to be quantified in order to improve their proxy-value.

Benthic foraminifera are used as environmental bioindicators especially in polluted environments where their sensitivity to pollutants may be expressed by a modification of their test structure or by a change in the composition of the assemblage. Studies of pollution effects on benthic foraminifera and of the possible use of these organisms as proxies were initiated by Resig (1960) and Watkins (1961), although pollution effects on foraminifera had been mentioned earlier by other workers (e.g., Zalesny, 1959). Throughout the last few years, many studies dealing with benthic foraminifera as bioindicators of coastal pollution have been carried out (review in Boltovskoy et al., 1991; Yanko et al., 1999; Samir, 2000; Scott et al, 2001; Debenay et al., 2001; Armynot du Châtelet et al., 2004; Bergin et al., 2006; Romano et al., 2008; Martins et al., 2010). An entire volume of the Journal of Foraminiferal Research was devoted to this problem in 1995. They concluded that foraminifera are very sensitive to pollution. Foraminifera should be applied as part of integrated programs of pollution monitoring, including chemical analysis of the contaminants. Their uses should include routine long-term surveillance programs, hazard assessment at specific discharge sites, and monitoring of the effectiveness of remedial actions (Debenay et al., 2000).

Foraminifera are known from the early Cambrian until today. The tests can be very abundant in marine sediments, they actually making up the bulk of several rocks. In the geological history foraminifera are important as biostratigraphic indicators in marine rocks, because they were abundant and diverse. Planktonic foraminifera are widespread and have had rapidly evolving lineages. These factors aid the inter-regional correlation of strata. They also help to reconstruct the paleo-climate because the ecological conditions while the lifetime of the organism can be red in the test (McGowran, 2005).

Benthic foraminifera are classified into orders based on their shell structure. Murray (1973) showed that relative proportions of the three most common benthic groups, when plotted on a ternary diagram, provide clues to the environments in which these organisms live. These three groups include the porcelaneous Miliolida; the calcareous perforate taxa that are now classified in several orders, the most important of which are the Rotaliida and the Buliminida; and the agglutinated taxa, which include the Lituolida, Trochamminida, and Textulariida. The Miliolida have been

important environmental indicators from the Carboniferous onward. They secrete imperforate shells of high magnesium calcite, which are most easily produced in warm, shallow, hypersaline waters saturated with $CaCO_3$ (Haynes, 1981). The agglutinated taxa, particularly those that produce organic or ferrigenous adhesives, generally dominate environments where sea water is undersaturated with respect to $CaCO_3$, such as estuarine, polar and deep-sea habitats. Finally, the perforate taxa have been able to adapt to a great range of environments. This order not only includes exceptionally eurytopic, opportunistic taxa, including *Ammonia* spp., but also highly specialized taxa such as algal symbiont-bearing forms (Hallock, 2000).

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Chapter 2

Distribution patterns of recent organic-walled dinoflagellate cysts in relation to environmental parameters in the Mediterranean Sea

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Abstract

To determine the relationship between the spatial dinoflagellate cyst distribution and oceanic environmental conditions, 34 surface sediments from the eastern and western Mediterranean Sea have been investigated for their dinoflagellate cyst content. Multivariate ordination analyses identified sea-surface temperature, chlorophyll-*a*, nitrate concentration, salinity, and bottom oxygen concentration as main observed factors affecting dinoflagellate cyst distribution in the region. Based on the relative abundance data, two associations can be distinguished that can be linked with major oceanographic settings. (1) An offshore eastern Mediterranean regime where surface sediments are characterized by oligotrophic, warm, saline surface water, and high oxygen bottom water concentrations (*Impagidinium* species, *Nematosphaeropsis labyrinthus, Pyxidinopsis reticulata* and *Operculodinium israelianum*). Based on the absolute abundance, temperature is positively related to the cyst accumulation of *Operculodinium israelianum*. Temperature does not form a causal factor influencing the accumulation rate of the other species in this association. *Impagidinium* species and *Nematosphaeropsis labyrinthus* show a positive relationship between cyst accumulation and nitrate availability in upper waters. (2) Species of association 2 have highest relative abundances in the western Mediterranean Sea, Strait of Sicily/NW Ionian Sea and/or the distal ends of the Po/Nile/Rhône River plumes. At these stations, surface waters are characterized by relative to the other regime higher productivity associated with lower sea-surface temperature, salinity, and lower bottom water oxygen concentrations (*Selenopemphix nephroides, Echinidinium* spp., *Selenopemphix quanta, Quinquecuspis concreta, Brigantedinium* spp. and *Lingulodinium machaerophorum*). Based on both the absolute and relative abundances, *Selenopemphix nephroides* is suggested to be a suitable indicator to trace changes in the trophic state of the upper waters. The distribution of *Lingulodinium machaerophorum* is related to the presence of river-influenced surface waters, notably the Nile River. We suggest that this species might form a suitable marker to trace past variations in river discharge, notably from the Nile.

Keywords: Mediterranean Sea; dinoflagellate cysts; temperature; productivity; preservation; eutrophication.

2.1 Introduction

Today the Mediterranean Sea is one of the most oligotrophic seas in the world (Yacobi et al., 1995). Unfortunately the increasing anthropogenic pressure on the region results in an growing problem of local eutrophication notably in coastal areas, especially near river mouths and in coastal lagoons (Carlier et al., 2008). Within the last decades the awareness about the socio-economic effects of eutrophication has increased and there is a rising demand for studies that provide accurate information about recent and past environmental changes that have taken place in the affected areas. A way to obtain this information is by establishing detailed palaeo-environmental reconstructions from time intervals prior and during the eutrophication events. Dinoflagellate cysts form a suitable tool to establish such reconstructions as their fossilized assemblages can reflect the environmental conditions of upper waters in detail (e.g. Pospelova et al., 2002; Dale, 2009). However, studies on the geographic distribution of dinoflagellate cysts have shown that, although there are some generalities, every aquatic system has a unique cyst association when studied in detail (e.g. Dale, 1996; de Vernal et al., 1997; Rochon et al., 1999; Devillers and de Vernal, 2000; de Vernal et al., 2001; Marret and Zonneveld, 2003; Pospelova et al., 2004; 2008; Radi et al., 2007; Holzwarth et al., 2007; Bouimetarhan et al., 2009). For an adequate reconstruction of palaeo-environmental changes in the past it is therefore a requirement to obtain detailed information about the local cyst distribution in surface sediments and its relationship to oceanographic and environmental conditions of the upper waters. Today there is little information available on the distribution of dinoflagellate cysts in recent sediments from the Mediterranean Sea. Although information about eutrophic areas in the Eastern Mediterranean has recently increased as a result of some detailed surveys in the Po-river plume, information about the modern cyst distribution in the oligotrophic parts of the Sea is practically lacking as it is restricted to surveys of few sites only or has not been published yet (Rubin et al., 2000; Sangiorgi et al., 2005; Giannakourou et al., 2005; Marret et al., 2008 at http://black.sealevel.ca/dinocystA3.jp; Zonneveld, et al., in press). Here we intent to increase this information by documenting the dinoflagellate cyst associations of 34 western and eastern Mediterranean surface sediment samples originating from different environments (Fig. 1). Most samples originate from the oligotrophic open

Mediterranean, whereas some sites are located in more mesotrophic/eutrophic environments at the most distal end of river plumes. Cyst distribution patterns are being compared to upper and bottom water characteristics. The relative abundance of dinoflagellate cysts is used to characterize different environmental regimes. Cyst accumulation rates are used to increase our understanding of the ecology of different species. This study forms a basis for further studies to use these cysts as bioindicators or proxies for eutrophication monitoring along the Mediterranean coast.

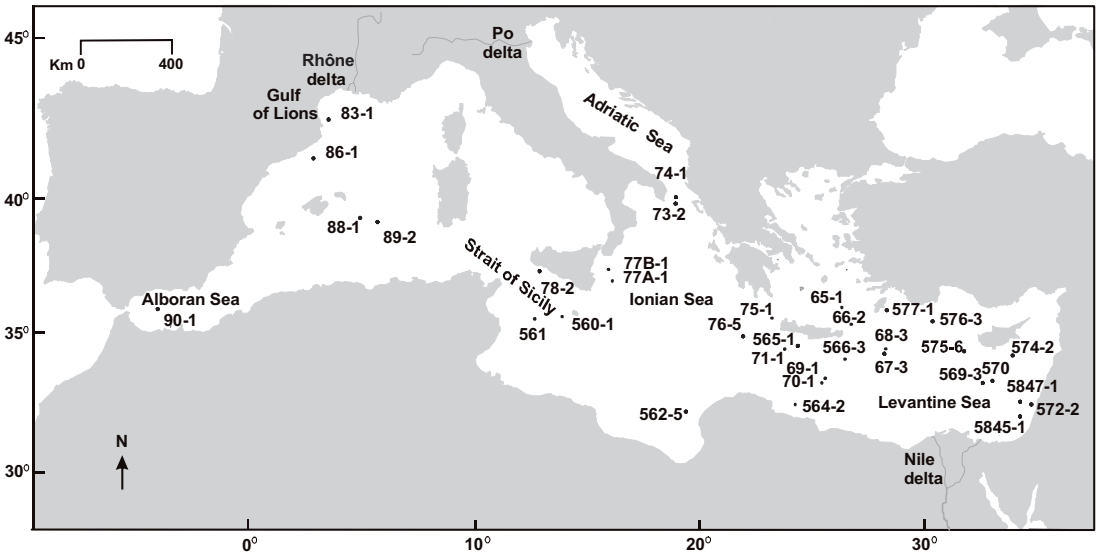

Figure 1. Map of the Mediterranean Sea showing the location of sampling sites.

2.2 Study area

2.2.1 Oceanography

The Mediterranean Sea is ~ 4000 km long, 1000 km wide and 1500 m deep (Candela, 1991). It is a land-locked marginal sea of the Atlantic Ocean and it is divided by the Strait of Sicily into two main sub-basins; the western and eastern Mediterranean (Fig. 1). It is a concentration basin with evaporation losses exceeding the freshwater input from precipitation and river runoff (e.g. Ludwig et al., 2009 and references therein). This in combination with winter cooling, results in the formation of deep and intermediate waters, notably in the north-western part of the Western and marginal basins of the Eastern Mediterranean Sea (e.g. Pinardi and Masetti, 2000; Tsimplis et al., 2006; Grbec et al., 2007). In turn, this results in a thermohaline-circulation pattern of the basin in which an eastward flow of surface waters originating from the Atlantic Ocean overlies a westward flow of intermediate and

deep waters (Fig. 2). Upper water circulation patterns are characterized by the presence of large-scale cyclonic gyres (Fig. 3A).

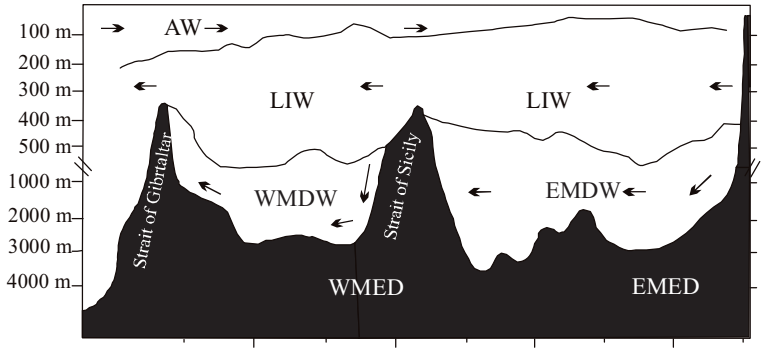

Figure 2. East - West cross section showing water mass circulation in the Mediterranean Sea (after Zavatarelli and Mellor, 1995). WMED=western Mediterranean Sea; EMED=eastern Mediterranean Sea; AW=Atlantic Water; LIW=Levantine Intermediate Water; EMDW=Eastern Mediterranean Deep Water; WMDW=Western Mediterranean Deep Water.

Atlantic waters entering at the Strait of Gibraltar undergo progressive modifications, becoming warmer and saltier in eastward direction and form Levantine Surface Water (LSW; Tsimplis et al., 2006). Mean annual temperature values of the surface water are lowest in the Gulf of Lions (~17°C) and highest in the south-eastern Levantine Sea where temperatures of more than 22°C are reached (Levitus and Boyer, 1994) (Fig. 4A). Salinity ranges from about 36.5 psu at the Strait of Gibraltar to more than 39.0 psu in the Levantine Sea (Levitus and Boyer, 1994) (Fig. 4B). Surface waters of both the Western and Eastern Mediterranean Sea are mainly oligotrophic resulting in low bioproductivity which is reflected by low chlorophyll-*a* concentrations (Psarra et al., 2000; Krom et al., 2003). However, there is a prominent west-east trophic gradient with surface waters becoming increasingly oligotrophic in eastward direction (Turley et al., 2000; Psarra et al., 2005) (Fig. 5). Nutrients and trace elements are mainly brought into the upper waters by local upwelling and river input. Nutrient-enriched upper water masses related to upwelling can be found in the Alboran Sea and along the Algerian coast as a result of the presence of gyres and eddies (Raimbault et al., 1993) (Figs. 4C, D, 5). The Alboran Sea surface circulation consists of a western and an eastern anticyclonic gyre causing upwelling of sub-suface nutrient rich waters along the northern limb of the western gyre and the development of a frontal system along the east of the eastern gyre (Tintoré et al., 1991).

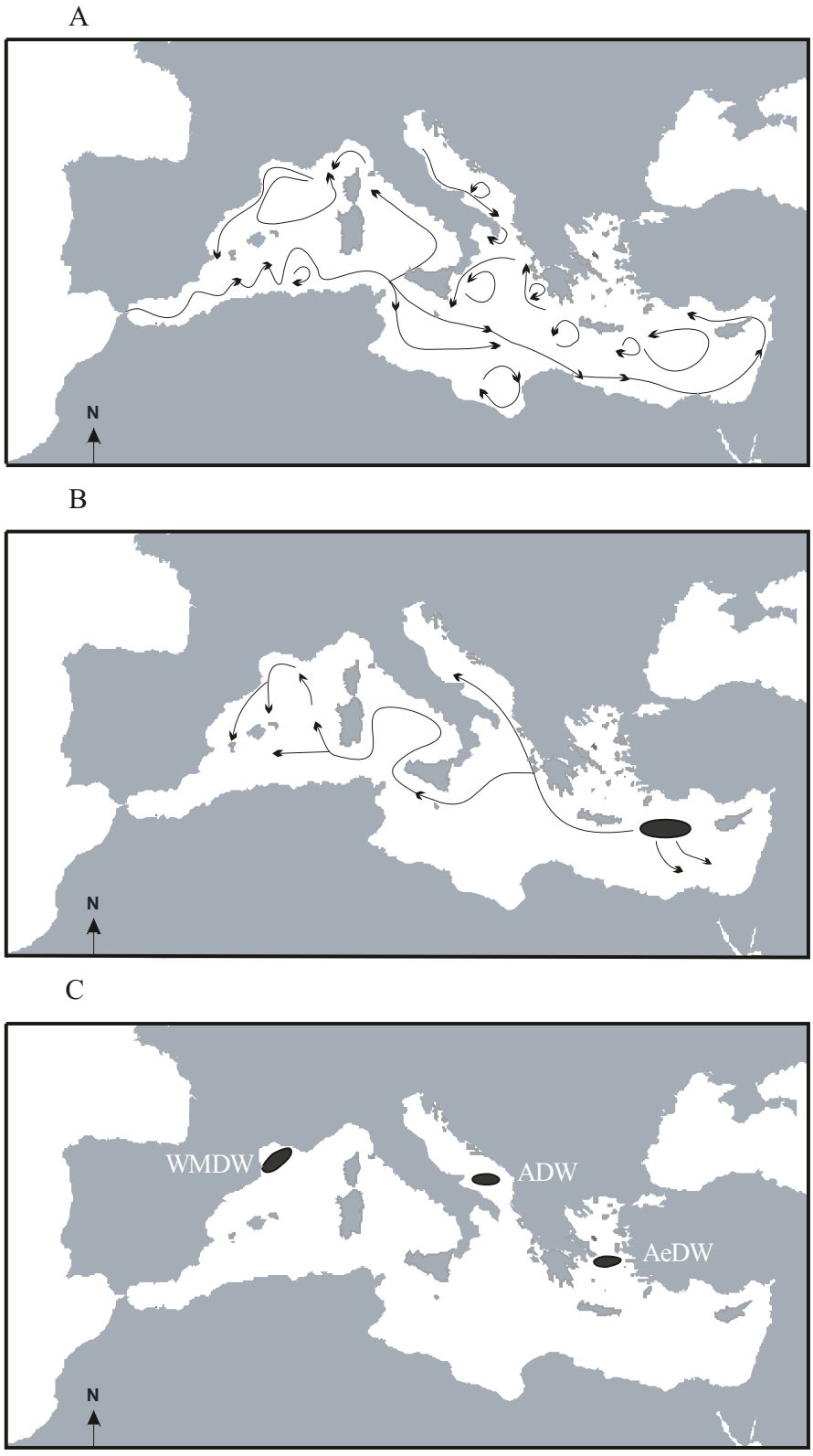

Figure 3. Water mass circulation in the Mediterranean Sea. (a) Schematic representation of the major features of the surface water circulation. (b) Circulation pattern of the Levantine Intermediate Waters (LIW). (c) Sites of deep water overturning (dense water formation) (modified after Pinardi and Masetti, 2000).

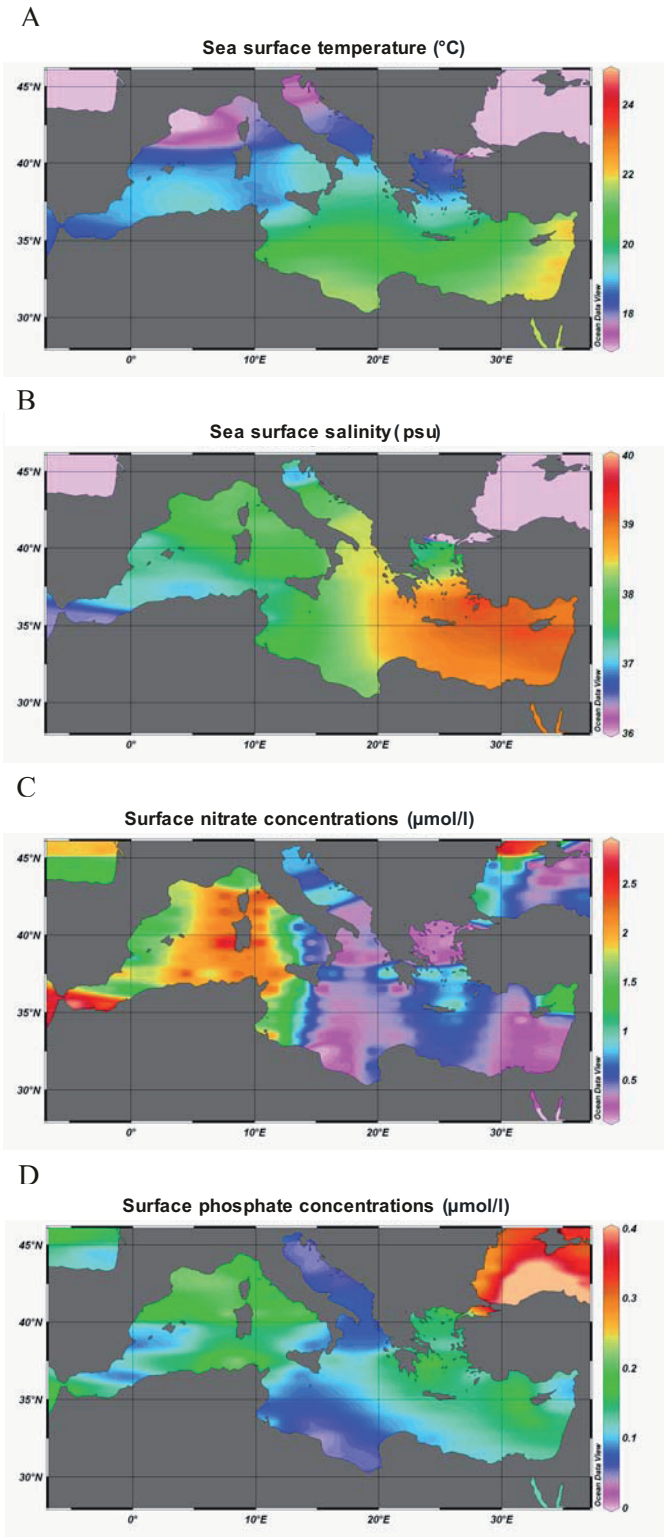

Figure 4. Map depicting annual surface temperature (°C) (A), annual surface salinity (psu) (B), annual surface nitrate concentrations (µmol/l) (C), and annual surface phosphate concentrations (µmol/l) (D) in the Mediterranean Sea based World Ocean Atlas (2005), http://ingrid.ldgo.columbia.edu/SOURCES/.LEVITUS94/.

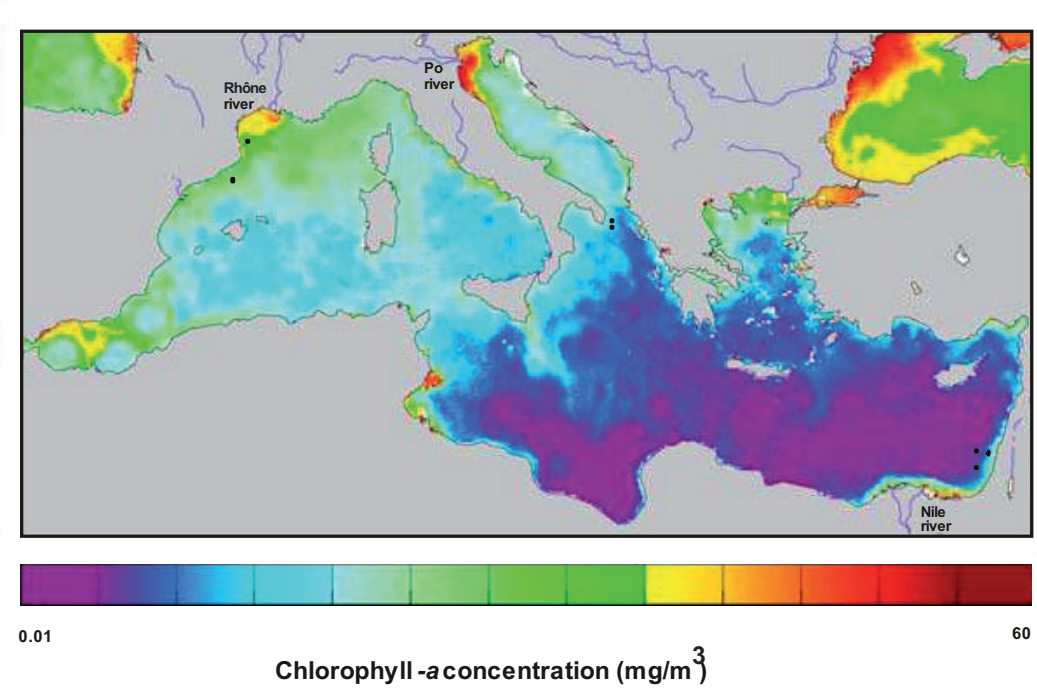

Figure 5. Map depicting the annual chlorophyll-*a* concentrations (mg/m^3) at the sea-surface based on http://reason.gsfc.nasa.gov/OPS/Giovanni/ocean.aqua.shtml.

Three major rivers draining into the Mediterranean Sea are the Rhône, Po, and Nile Rivers. Although the Nile has the largest catchment area of all rivers entering the Mediterranean Sea, the construction of the Aswan High Dam in 1964 resulted in a major reduction in discharge from about 4-8 * 10^{10} m^3 yr^{-1} previous to construction (Abu El Ella, 1993) to about 6 - 15 * 10^9 m^3 yr^{-1} today (Wadie, 1984; Nixon, 2003). As a result, today the Rhône and the Po alone account for about one third of the average freshwater input in the Mediterranean Sea (Ludwig et al., 2009). Due to the anti-clockwise surface circulation in the Adriatic Sea induced by Coriolis forcing, the nutrient-rich Po River discharge water is pressed against the western coastal margin of the basin (e.g. Lee et al., 2007). As a consequence, a band of enhanced productivity reflected by high chlorophyll-*a* concentration in surface waters, can be observed along the whole western margin of the Adriatic Sea, the Strait of Otranto, around the Calabrian Peninsula into the Golfo di Taranto (Fig. 5). Along the Egyptian and Arabian Peninsula coasts a small band of enhanced upper-ocean productivity can be found related to the Nile River outflow (Fig. 5).For practical

reasons we refer in this paper to these bands of enhanced upper ocean chlorophyll-*a* concentrations as representing the Po and Nile River discharge plumes.

Eutrophic surface waters are found in coastal areas as well as where enclosed bays, local rivers, and estuaries receive nutrient loads from domestic and industrial wastewaters (Karydis and Chatzichristofas, 2003) (Fig. 5).

Subsurface waters consist of Intermediate Waters that are mainly formed in the northern Levantine Basin as a result of winter cooling (e.g. Lascaratos et al., 1999; Pinardi and Masetti, 2000). It spreads throughout the Mediterranean Sea at a depth between 200 and 600 m eventually entering the Atlantic Ocean through the Gibraltar Strait (Figs. 2, 3B).

Below this intermediate water, deep-water masses are found that are produced separately in the western and eastern Mediterranean Sea (Pinardi and Masetti, 2000) (Fig. 3C). A change in the Eastern Mediterranean Sea circulation has been observed around the early 1990s characterized as the Eastern Mediterranean Transient (EMT). Prior the EMT, thermohaline circulation in the Eastern Mediterranean Sea involved only one deep water formation zone in the Adriatic Sea. The new observations indicate that the Cretan/Aegean Sea has become an additional driving engine of the intermediate and deep water displacing the older Eastern Mediterranean Deep Water (e.g. Lascaratos et al., 1999; Tsimplis et al., 2006). The formation of deep water results in a well ventilated bottom water layer. Bottom water oxygen concentrations are high throughout the Mediterranean Sea with exception of the eutrophicated coastal areas. In contrast to upper water conditions, there is no clear east/west gradient (Fig. 6).

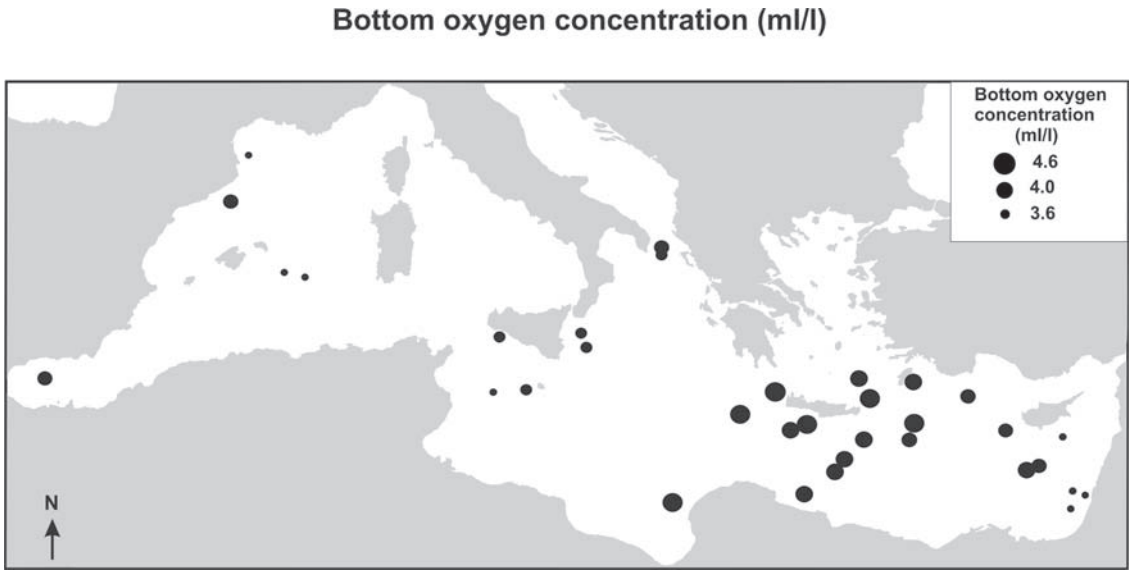

Figure 6. Annual bottom oxygen concentrations (ml/l) at the studied stations.

2.3 Material and methods

Thirty-four surface sediment samples have been collected by multicorer during the RV *Meteor* expeditions M40/4 (Hieke et al., 1999), M44/3 (Pätzold et al., 2000), and M51/3 (Hemleben et al., 2001) (Fig. 1). The uppermost centimetre (0 – 1 cm) of each core was analysed. Literature based information on tephra chronology, ^{210}Pb dating, and ^{14}C dating allowed the determination of sedimentation rates at 9 stations. Sedimentation rates varied from 2.5 cm/kyr (St. 71-1, central Eastern Mediterranean) to 27 cm/kyr (St. 572-2, distal end of the Nile plume) indicating that upper cm sediments represent 37 – 400 years of sedimentation history (Table 1 and references therein).

Table 1. Sample coordinates, water depths, sampling cruises and total cyst counted and the number of cysts per gram. Western part and Strait of Sicily/NW Ionian Sea stations are marked with an asterix. SR = sedimentation rate, DBD = dry bulk density (g/cm^3).

Station	Cruise	Latitude	Longitude	Depth (m)	Count	Cysts/ g	DBD	SR (cm/kyr)
70-1	M40/4	33◦42.81′N	24◦42.11′E	2107	261	256		
71-1	M40/4	34◦48.63′N	23◦11.64′E	2787	109	108	0.033	2.5 (Heike et al., 1999)
77A-1*	M40/4	37◦00.18′N	16◦09.38′E	2719	204	366		
77B-1*	M40/4	37◦23.66′N	16◦00.37′E	2519	210	230		
73-2*	M40/4	39◦31.70′N	18◦58.34′E	838	200	200	0.033	10 (Heike et al., 1999)
76-5	M40/4	35◦13.66′N	21◦30.02′E	3376	183	177		
83-1*	M40/4	42◦27.69′N	03◦29.64′E	911	189	193	0.067	10 (Heike et al., 1999)
78-2*	M40/4	37◦02.12′N	13◦11.39′E	471	342	408		
66-2	M40/4	35◦36.00′N	25◦54.33′E	560	260	302		
65-1	M40/4	36◦08.75′N	25◦33.81′E	828	406	483	0.005	8.75 (Heike et al., 1999)
69-1	M40/4	33◦51.58′N	24◦51.44′E	2155	166	231		
67-3	M40/4	34◦48.81′N	27◦17.76′E	2157	629	749	0.005	3.5 (Frydasa and Hemleben, 2007)
75-1	M40/4	35◦48.62′N	22◦40.22′E	1019	322	304		
68-3	M40/4	34◦41.37′N	27◦16.24′E	2120	278	271	0.013	3 (Heike et al., 1999)
74-1*	M40/4	39◦56.74′N	18◦59.85′E	911	439	437		
88-1*	M40/4	38◦56.26′N	4◦36.07′E	2446	941	929	0.005	5.9 (Shipboard Scientific party, 1996a, b)
86-1*	M40/4	41◦12.36′N	02◦50.02′E	1268	675	654		
90-1*	M40/4	36◦01.00′N	01◦56.99′W	1955	631	621	0.005	10.4 (Shipboard Scientific party, 1996a, b)
89-2*	M40/4	38◦45.05′N	05◦20.22′E	2782	374	787		
5845-1	M44/3	32◦19.29′N	34◦09.90′E	1284	114	57		
5847-1	M44/3	32◦49.23′N	34◦09.84′E	1532	241	268		
572-2	M51/3	32◦44.51′N	34◦38.94′E	892	258	292	0.005	27 (Hamann et al., 2008)
570	M51/3	33◦31.81′N	32◦59.47′E	2064	250	242		
577-1	M51/3	35◦54.10′N	28◦30.99′E	4285	127	62		
560-1*	M51/3	35◦51.27′N	14◦06.20′E	996	213	247		
575-6	M51/3	34◦31.64′N	31◦47.15′E	2337	392	347		
566-3	M51/3	34◦28.00′N	25◦39.98′E	1339	293	254		
561*	M51/3	35◦47.87′N	12◦59.53′E	485	416	529		
576-3	M51/3	35◦34.40′N	30◦27.67′E	1275	313	311		
564-2	M51/3	33◦00.04′N	23◦37.77′E	1476	254	218		
565-1	M51/3	34◦55.26′N	23◦44.58′E	1048	369	360		
569-3	M51/3	33◦27.18′N	32◦34.94′E	1294	316	314		
574-2	M51/3	34◦26.90′N	33◦51.66′E	1171	288	283		
562-5	M51/3	32◦46.45′N	19◦11.46′E	1391	315	293		

2.3.1 Palynological Analyses

For organic-walled dinoflagellate cyst preparation, samples were dried at 60°C for 24 h, and weighed. Cold 10% HCl was added to remove carbonates, followed by repeated washes with distilled water until all dissolved carbonate was removed and the sample was neutral. The material was allowed to settle down undisturbed for 6-7 h between the washings. To remove silicates, the material was treated with 40% cold HF, agitated for 2 h, and remained in HF for additional two days without agitation. After HF-treatment, the material was neutralised as before, i.e. the samples were decanted three times with a minimum interval of 6-7 h. The neutral material was homogenised (i.e. shaken for 2 min), sonified for l-2 min to disaggregate, and sieved over a 20 μm stainless steel sieve to retain the > 20 μm fraction. Each residue was centrifuged in a glass tube (8 min, 3300 rpm) and transferred to a 1.5 ml Eppendorf tube. After centrifuging again (6 min, 3300 rpm) the material was concentrated to 0.5 ml in an Eppendorf tube. 50 μl of the homogenised sample residue was put on a slide with an Eppendorf pipette. The material was embedded in glycerine jelly and insulated from air by paraffin wax. Whole slides were analysed on their dinoflagellate cyst content by using light microscope at magnification of 400 X. The taxonomy is according to Marret and Zonneveld (2003), *Echinidinium* spp. includes all spiny brown cysts that could not be identified on species level. *Brigantedinium* spp. includes all smooth-walled spherical brown cysts. A minimal amount of 100 cysts per slide have been counted when possible (up to 941 specimens in rich samples, Table 1).

In addition, the cyst accumulation rate was calculated according to the following formula:

$$AR\ (c/cm^2/kyr) = Cyst/g\ (c/g) * DBD\ (g/cm^3) * SR\ (cm/kyr)$$

Where AR = accumulation rates, Cyst/g = amount of cysts per gram dry sediment, DBD = dry bulk density, SR = sedimentation rate. DBD was measured by weighing the amount of dry sediment (g) occupied one cm^3.

2.3.2 Statistical Methods

To assess the nature of variability of dinoflagellate cyst assemblages, a Detrended Correspondence Analysis (DCA) was carried out using the software package CANOCO version 4.02 including data from all samples. Excluding the data from the few samples that contained less that 100 cysts did not change the outcome of the statistical analyses. As result these samples have not been excluded from the analyses. The relation between chosen environmental data and dinoflagellate cyst species abundances were assessed with a multivariate ordination technique of Canonical Correspondances Analysis (CCA). Two DCA and CCA analyses were carried out. The first run (DCA1 and CCA1) included the relative abundance of dinoflagellate cyst taxa, the second analyses (DCA2 and CCA2) are based on their accumulation rates at 9 of the 34 stations. For every analysis a Monte Carlo permutation test was performed to test the significance of each parameter. Rare taxa were excluded from all statistical analyses (Table 5). Background information of the methods can be found in Jongman et al. (1987) and Ter Braak (1986; 1987a,b).

Seasonal sea-surface temperature (T), sea-surface salinity (S), nitrate (N), and phosphate (P) concentration at 0 m and annual bottom water oxygen concentration (Ox_{an}) were obtained from the World Ocean Atlas (2005). These data were measured on 1 degree grid scale. Seasonal chlorophyll-*a* concentrations (chl) data were derived from the online remote sensing observations, http://reason.gsfc.nasa.gov/OPS/Giovanni/ocean.aqua.shtml, on a resolution of 0.1 degree around the sample site compiled from data measured by satellites between December 2002 – December 2008. Seasons are defined as such: winter: January – March, spring: April – June, summer: July – September, and autumn: October – December. The values of environmental parameters are given in Table 2.

Table 2a. Seasonal surface temperature (°C), salinity (psu), nitrate (µmol/l), phosphate (µmol/l), seasonal chlorophyll-*a* concentrations (mg/m^3), and annual bottom oxygen concentrations (ml/l). Abbreviations: T_w, T_{sp}, T_s, and T_{au}: Sea surface temperature during winter, spring, summer, and autumn, S_w, S_{sp}, S_s, and S_{au}: Sea surface salinity during winter, spring, summer, and autumn, N_w, N_{sp}, N_s, and N_{au}: Sea surface nitrate concentration during winter, spring, summer, and autumn.

Stations	T_w	T_{sp}	T_s	T_{au}	S_w	S_{sp}	S_s	S_{au}	P_w	P_{sp}	P_s	P_{au}	N_w	N_{sp}	N_s	N_{au}
70-1	16.48	19.22	25.18	21.19	38.79	38.71	38.99	39.04	0.16	0.05	0.12	0.12	0.5	0.4	0.1	1
71-1	16.07	18.91	24.68	20.58	38.83	38.80	38.99	38.99	0.16	0.07	0.16	0.12	1	0.6	0.1	0.5
77A-1	14.94	18.61	25.25	19.86	38.13	38.02	37.98	38.05	0.21	0.17	0.16	0	0.2	0.9	0.6	1
77B-1	14.94	18.61	25.25	19.86	38.13	38.02	37.98	38.05	0.21	0.17	0.16	0	0.2	0.9	0.6	1
73-2	14.39	18.31	24.59	19.03	38.47	37.47	38.37	38.38	0.09	0.12	0.06	0.01	1	0.4	0.7	0.7
76-5	15.82	18.91	25.08	20.55	38.69	38.69	38.87	38.74	0.17	0.09	0.16	0.12	0.4	0.8	0.3	0.3
83-1	12.69	15.84	21.23	16.58	38.11	37.59	37.69	37.7	0.23	0.1	0.37	0.15	2.8	1	2.3	0.7
78-2	14.44	17.14	24.11	19.36	37.20	37.15	37.35	37.48	0.21	0.15	0.07	0.16	0.2	0.3	0.3	4.5
66-2	16.50	19.38	24.14	20.50	38.94	39.04	39.16	39.12	0.15	0.15	0.12	0.16	1	0.7	0.2	0.8
65-1	16.08	18.96	23.45	19.62	39.02	39.17	39.09	39.04	0.12	0.2	0.16	0.13	1	0.4	0.1	0.2
69-1	16.48	19.22	25.18	21.19	38.79	38.71	38.99	39.04	0.16	0.05	0.12	0.12	0.5	0.4	0.1	1.2
67-3	16.47	19.54	24.85	20.89	38.98	38.96	39.17	39.18	0.16	0.11	0.11	0.17	0.9	0.5	0.1	0.9
75-1	15.86	18.88	24.71	20.41	38.68	38.71	38.83	38.72	0.17	0.09	0.2	0.14	0.8	0.8	0.4	0.5
68-3	16.47	19.54	24.85	20.89	38.98	38.96	39.17	39.18	0.16	0.11	0.11	0.17	0.9	0.5	0.1	0.9
74-1	14.39	18.31	24.59	19.03	38.17	37.47	38.07	37.38	0.09	0.12	0.06	0.01	1.6	0.9	1.3	1.2
88-1	14.57	18.04	24.76	19.32	37.28	37.17	37.12	37.26	0.16	0.14	0.09	0.05	3.8	0.8	0	3.5
86-1	12.62	16	22.23	16.91	38.28	37.86	37.81	37.53	0.21	0.13	0.25	0.1	2.8	0.7	1.2	0.9
90-1	14.9	17.84	23.64	18.49	37.14	37.03	36.92	37.08	0.26	0.04	0.03	0.02	1.6	0.2	0.3	4.4
89-2	14.57	17.91	24.57	19.3	37.25	37.18	37.21	37.34	0.21	0.13	0.11	0.09	2.9	0.6	0.1	4.4
5845-1	17.43	20.78	26.99	23.07	38.93	39.06	39.19	39.26	0.17	0.09	0.15	0.18	0.8	0.4	0.6	0.4
5847-1	17.43	20.78	26.99	23.07	38.93	39.06	39.19	39.26	0.17	0.09	0.15	0.18	0.7	0.8	0.6	0.7
572-2	17.43	20.78	26.99	23.07	38.93	39.06	39.09	38.26	0.17	0.09	0.15	0.18	0.9	0.8	0.8	1
570	17.13	20.53	26.56	22.45	38.96	38.97	39.2	39.26	0.2	0.09	0.18	0.21	0.2	0.3	0.3	0.1
577-1	16.21	19.47	24.64	20.42	39.04	39.03	39.24	39.23	0.16	0.13	0.1	0.18	0.9	0.8	0	0.4
560-1	15.63	18.76	25.54	20.91	37.72	37.58	37.72	37.96	0.16	0.09	0.05	0.02	0	0.4	0	2.1
575-6	16.57	20.07	26.03	21.61	39.19	39.12	39.38	39.42	0.22	0.09	0.17	0.23	0	0.3	0	0.1
566-3	16.33	19.21	24.66	20.81	38.96	38.97	39.16	39.14	0.17	0.08	0.14	0.15	1.3	0.5	0.1	0.8
561	15.4	18.56	25.32	20.76	37.56	37.47	37.64	37.87	0.15	0.08	0.05	0.03	0	0.3	0	3.8
576-3	16.3	19.86	25.78	21	39.02	38.97	39.2	39.23	0.22	0.1	0.12	0.19	0.2	0.8	0.1	0.3
564-2	16.34	19.02	25.14	21.01	38.71	38.6	38.9	38.98	0.15	0.04	0.11	0.1	0.5	0.6	0.1	0.8
565-1	16.07	18.91	24.68	20.58	38.83	38.8	38.99	38.99	0.16	0.07	0.16	0.12	1	0.6	0.1	0.5
569-3	17.13	20.53	26.56	22.45	38.96	38.97	39.2	39.26	0.2	0.09	0.18	0.21	0.2	0.3	0.3	0.1
574-2	17.16	20.72	27.02	22.37	38.89	39.08	39.25	39.41	0.12	0.06	0.15	0.11	0.1	0	0	1.4
562-5	16.74	19.31	26.42	21.68	38.31	38.25	38.7	38.59	0.13	0.05	0.04	0.05	0	1.1	0.7	0.2

Table 2b. P_w, P_{sp}, P_s, and P_{au}: Sea surface phosphate concentration during winter, spring, summer, and autumn, chl_w, chl_{sp}, chl_s, and chl_{au}: Sea surface chlorophyll-*a* during winter, spring, summer, and autumn, and Ox_{an}: Annual bottom oxygen concentration.

Stations	Ox_{an}	Chl_w	Chl_{sp}	Chl_s	Chl_{au}
70-1	4.41	0.14	0.09	0.06	0.09
71-1	4.45	0.16	0.10	0.08	0.11
77A-1	4.14	0.23	0.16	0.10	0.15
77B-1	4.12	0.24	0.19	0.12	0.16
73-2	4.12	0.22	0.26	0.22	0.18
76-5	4.54	0.16	0.10	0.07	0.12
83-1	3.97	0.41	0.40	0.20	0.30
78-2	4.17	0.42	0.25	0.16	0.23
66-2	4.5	0.18	0.13	0.08	0.13
65-1	4.4	0.19	0.13	0.09	0.12
69-1	4.45	0.14	0.09	0.06	0.09
67-3	4.5	0.13	0.14	0.08	0.12
75-1	4.57	0.17	0.11	0.08	0.12
68-3	4.32	0.13	0.14	0.08	0.12
74-1	4.22	0.22	0.27	0.13	0.28
88-1	4	0.36	0.16	0.09	0.19
86-1	4.2	0.56	0.39	0.14	0.29
90-1	4.2				
89-2	4.1	0.35	0.15	0.09	0.19
5845-1	3.9	0.17	0.19	0.16	0.11
5847-1	3.81	0.17	0.19	0.17	0.11
572-2	3.67	0.18	0.20	0.19	0.16
570	4.33	0.14	0.08	0.05	0.08
577-1	4.35	0.22	0.13	0.09	0.13
560-1	4.13	0.21	0.12	0.09	0.13
575-6	4.3	0.15	0.10	0.07	0.09
566-3	4.4	0.15	0.10	0.07	0.10
561	4.1	0.24	0.13	0.09	0.14
576-3	4.3	0.20	0.11	0.08	0.11
564-2	4.43	0.15	0.08	0.06	0.09
565-1	4.55	0.15	0.11	0.07	0.10
569-3	4.34	0.14	0.08	0.05	0.08
574-2	4	0.16	0.09	0.07	0.10
562-5	4.5	0.15	0.08	0.06	0.09

2.4 Results

2.4.1 Dinoflagellate Cyst Distribution and Assemblages

Cyst concentrations from open Mediterranean sites are low. The total dinoflagellate cyst concentrations vary by an order of magnitude, from 57 cysts g^{-1} to 929 cysts g^{-1} (Table 1). A marked difference in associations can be observed between the eastern part of the Mediterranean and the western part. Generally, higher concentrations of dinoflagellate cysts are found in the the western part compared to eastern sites (Table 1, 5).

Dinocyst assemblages are dominated by *Impagidinium aculeatum* that can form up to 67% of the assemblage. Other species that occur in high amounts are *Spiniferites* spp. (52.9%) and *Brigantedinium* spp. (51%). The majority of cyst types found in the

Mediterranean Sea belong to the order Gonyaulacales. They make up the complete association especially in the offshore eastern Mediterranean stations. Heterotrophic taxa are dominant in the western Mediterranean and Strait of Sicily/NW Ionian Sea (Table 5). Based on the visual examination of the dataset two groups of species can be distinguished. Group 1 consists of species that have their highest relative abundances in, or are restricted to the offshore eastern Mediterranean. This group is characterized by the gonyaulacoids phototrophic taxa: *Impagidinium aculeatum, Impagidinium sphaericum, Impagidinium paradoxum, Impagidinium patulum, Impagidinium plicatum, Impagidinium* spp., *Operculodinium israelianum, Pyxidinopsis reticulata* and *Nematosphaeropsis labyrinthus* (Figs. 7, 8, 9A). Species of group 2 have their highest relative abundances in, or are restricted to the western Mediterranean, Strait of Sicily/NW Ionian Sea and/or the most distal end of river discharge plumes. This group is characterized by the heterotrophic taxa *Selenopemphix nephroides, Echinidinium* spp., *Selenopemphix quanta, Quinquecuspis concreta* and *Brigantedinium* spp., and the phototrophic taxon *Lingulodinium machaerophorum* (Figs. 9B, C, D, 10A, B, C). *Echinidinium* spp. and *Selenopemphix quanta* are restricted to the distal ends of the regions influenced by maximal river discharge of the Rhône, Po, and Nile and around Sicily (Figs. 9C, D). *Selenopemphix nephroides* and *Quinquecuspis concreta* are more abundant in sediments of the most distal plume ends of the Rhône and/or Po River and in the western Mediterranean Sea around Sicily (Figs. 9B, 10A). *Brigantedinium* spp. has a wide distribution in the Mediterranean basin. Highest relative abundances are found at the distal end of Rhône River plume and around Sicily (Fig. 10B). *Lingulodinium machaerophorum* has its highest relative abundance in sites located at the distal part of the Nile River plume (Fig. 10C).

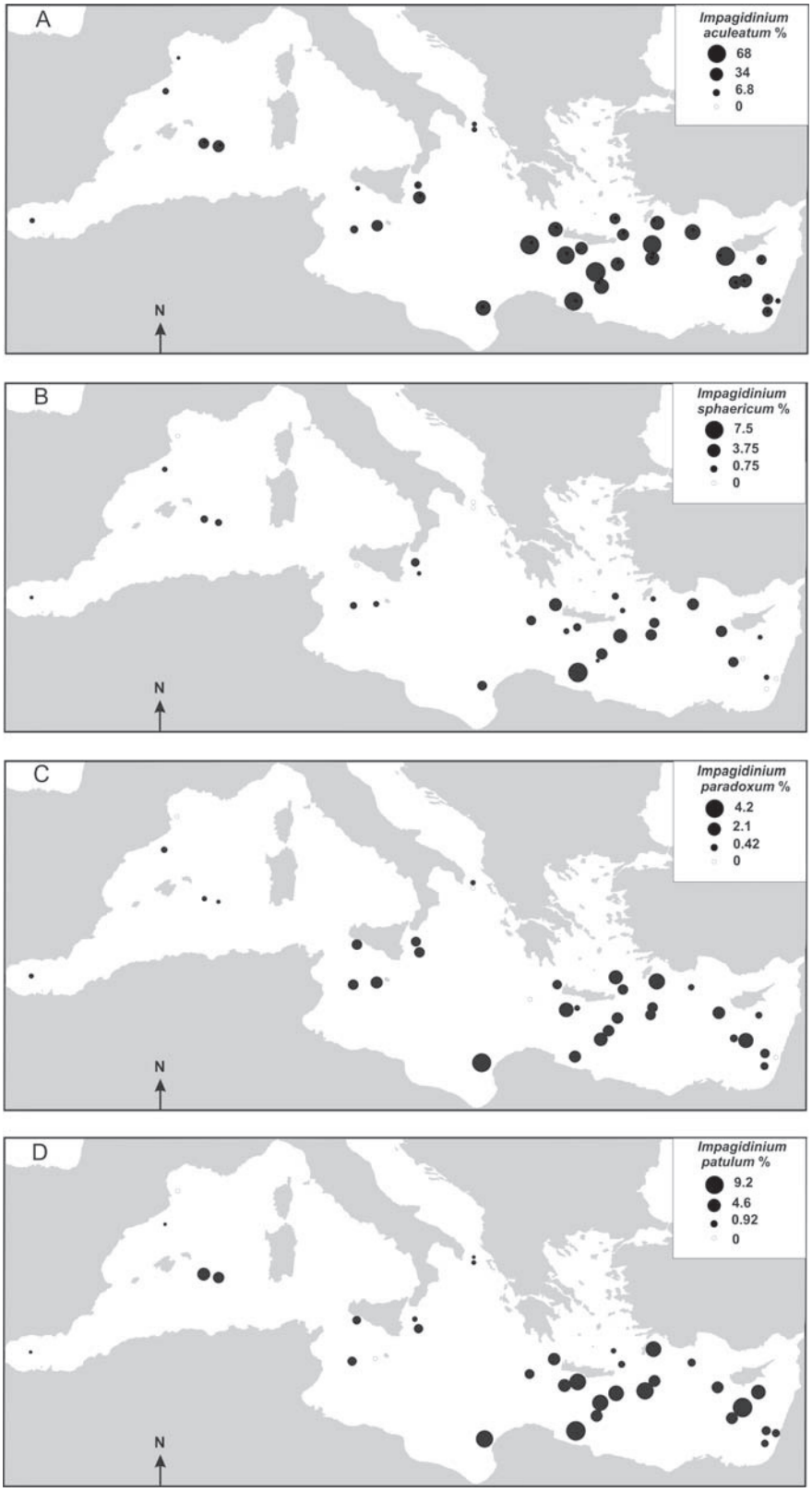

Figure 7. Map depicting the relative abundances of *Impagidinium aculeatum* (A), *Impagidinium sphaericum* (B), *Impagidinium paradoxum* (C), and *Impagidinium patulum* (D).

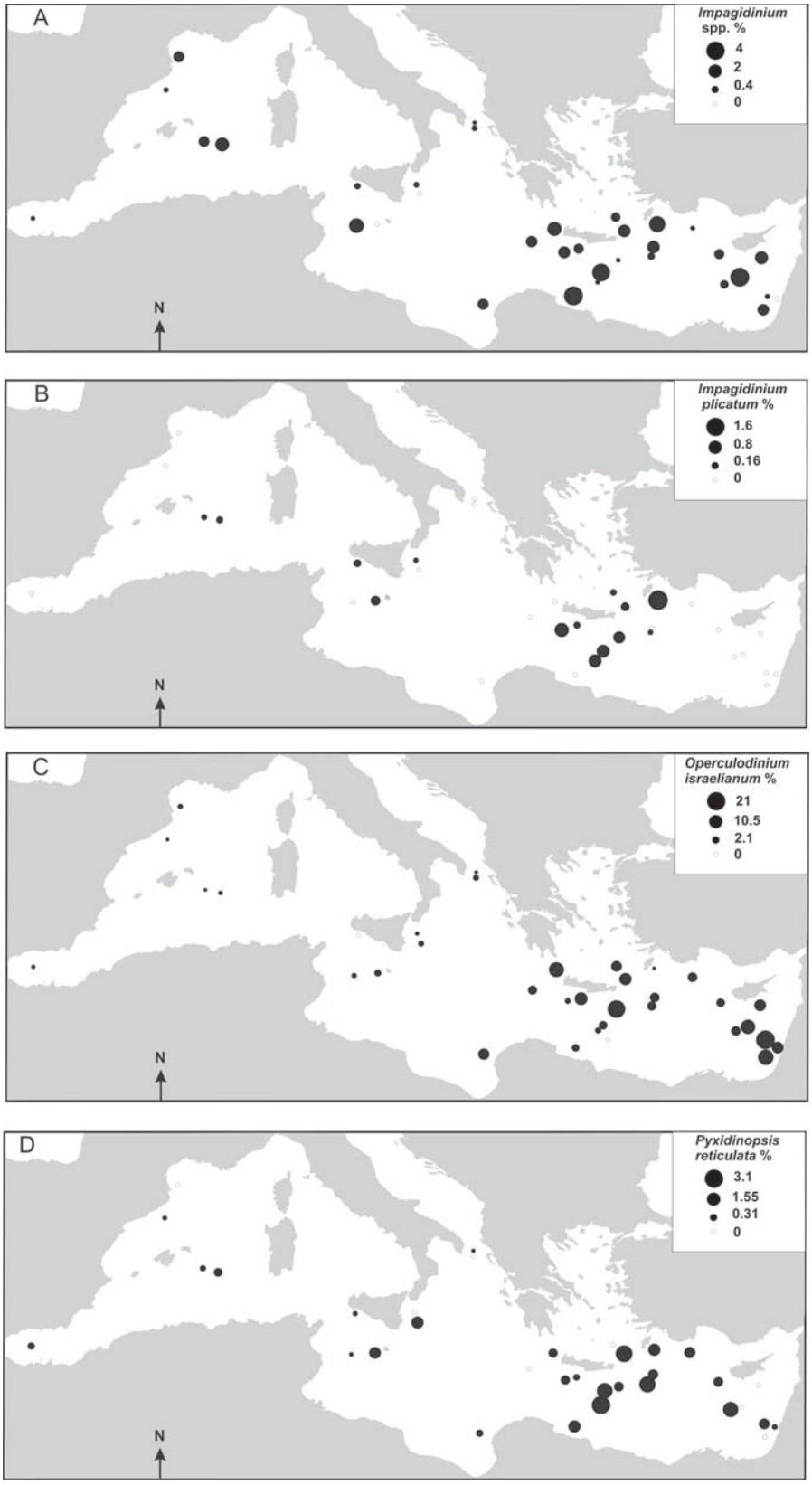

Figure 8. Map depicting the relative abundances of *Impagidinium* spp. (A), *Impagidinium plicatum* (B), *Operculodinium israelianum* (C), and *Pyxidinopsis reticulata* (D).

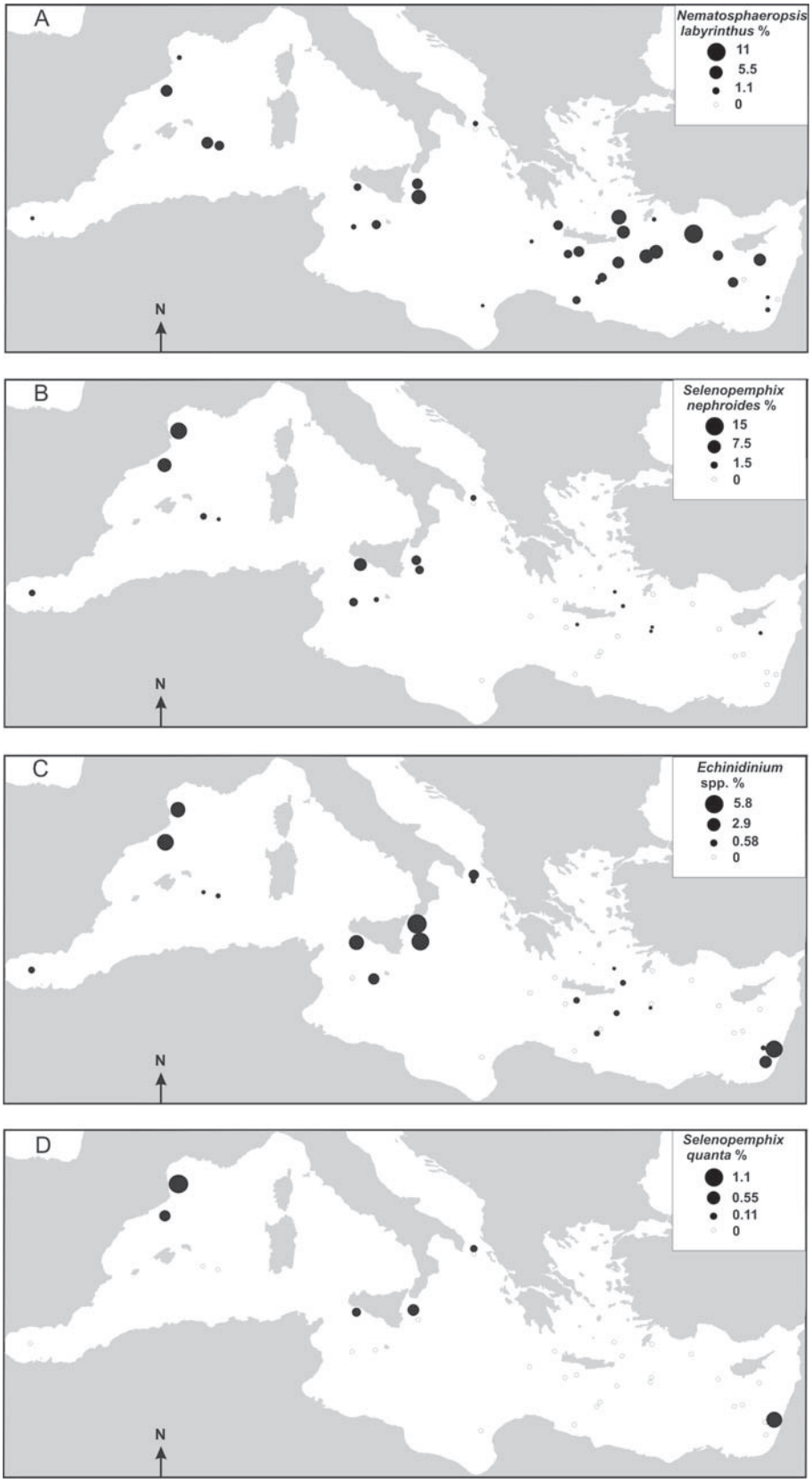

Figure 9. Map depicting the relative abundances of *Nematosphaeropsis labyrinthus* (A), *Selenopemphix nephroides* (B), *Echinidinium* spp. (C), and *Selenopemphix quanta* (D).

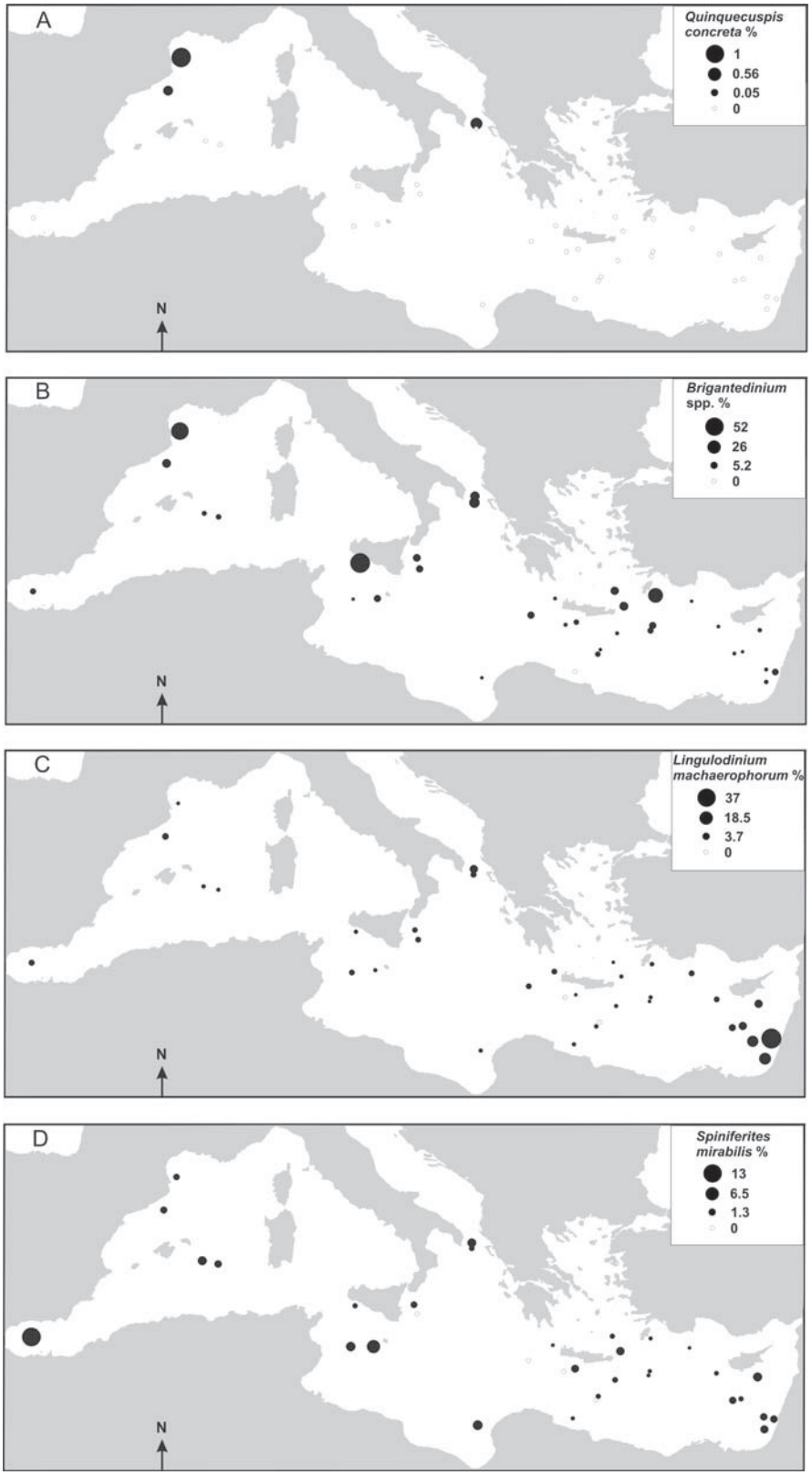

Figure 10. Map depicting the relative abundances of *Quinquecuspis concreta* (A), *Brigantedinium* spp. (B), *Lingulodinium machaerophorum* (C), and *Spiniferites mirabilis* (D).

2.4.2 Detrended Correspondence Analysis (DCA1)

The first and second DCA1 axes represent 32.6% and 6.9% of the variance in the data set respectively. The length of the first gradient, which was given in standard deviation (S.D.) units is 2.3, indicating the unimodal character of the data set. Higher axes explain less than 6% and are not taken into account. The DCA1 diagram of stations shows that the samples are arranged in two groups along the first axis, which obviously represents a variable or a combination of variables that show an E–W oriented gradient (Fig. 11). The first group of samples ordinated at the most positive right side of the first DCA1 axis, origin from the eastern offshore Mediterranean areas. Surface waters at these stations are characterized by high temperature, and salinity, as well as low productivity. Bottom waters at these sites have relatively high oxygen concentrations. The second group is ordinated at the left side of the first axis and consists of western Mediterranean stations, stations from the Strait of Sicily and NW Ionian Sea, and shallower Mediterranean stations located at the most distal end of the river plumes of the Nile, Po, and Rhône. Surface waters at these stations are characterized by relative lower temperature and salinity, as well as high productivity. Bottom waters contain relatively low oxygen concentration. Along the second axis, no clear geographical pattern is visible (Fig. 11).

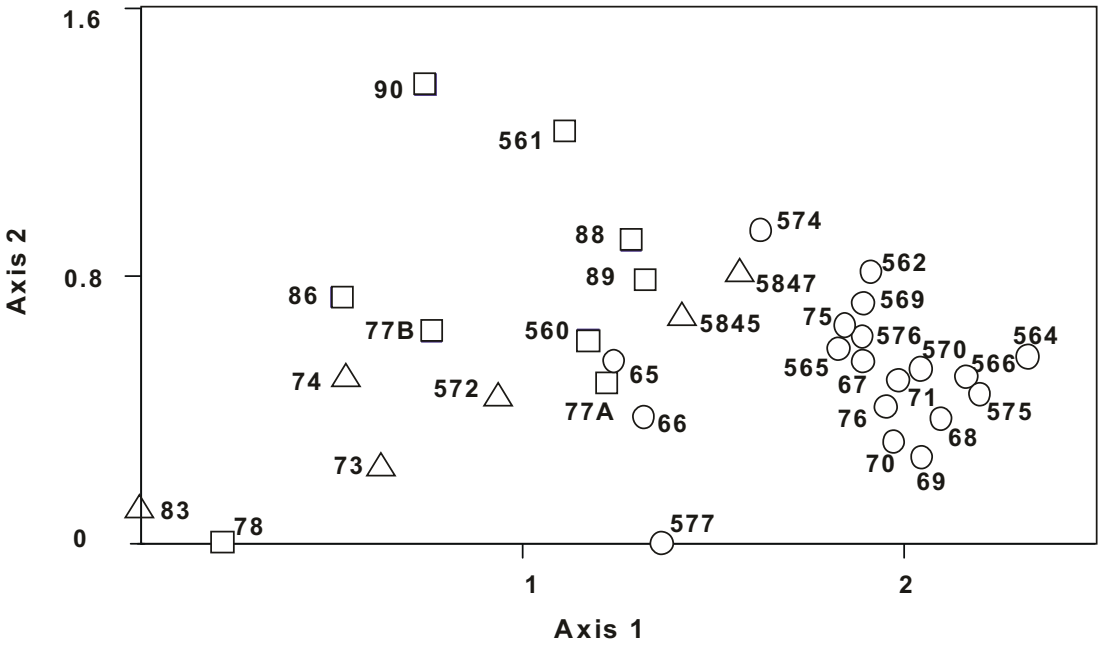

Figure 11. ordination diagram of DCA1 depicting sample positions. Squares represent samples from western Mediterranean and Strait of Sicily/NW Ionian Sea,

circles represent samples from eastern offshore Mediterranean, and triangles represent samples at the most distal end of river plumes in the Mediterranean Sea (Nile, Po, and Rhône).

The phototrophic taxa can be clearly distinguished from heterotrophic taxa. The species ordinated at the most positive part of the first axis are all known to be phototrophic, whereas those ordinated at the left side of the first axis belong to heterotrophic organisms (Fig. 12).

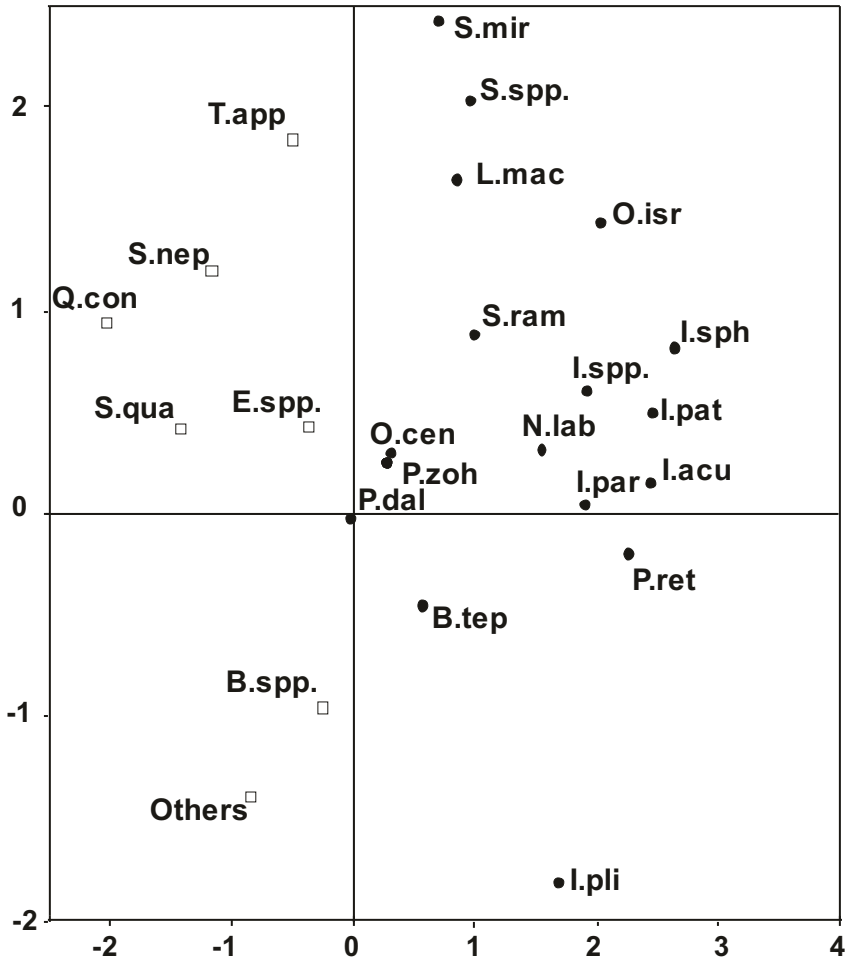

Figure 12. DCA1 diagram showing variance in the species distribution. Circles represent phototrophic taxa and squares represent heterotrophic taxa. For abbreviations see Table 6.

2.4.3 Dinoflagellate Cyst Groups Based on Relative Abundance Using Canonical Correspondence Analysis (CCA1)

The results of Monte Carlo Permutation tests show that T in all seasons, N_s, N_{au}, N_w, chl_w, S_{sp}, S_w, and Ox_{an} vary significantly in relationship to the relative cyst distribution (F value > 1) (Table 3). All other environmental variables do not significantly change in relationship to the spatial distribution of the individual cysts species. Winter temperature (T_w) forms the most important environmental parameter corresponding to 23% of the variation within the dataset. Sea surface temperature (SST), sea surface salinity (SSS), and bottom water oxygen gradients point into more or less the same direction, opposite to the productivity gradient (Fig. 13).

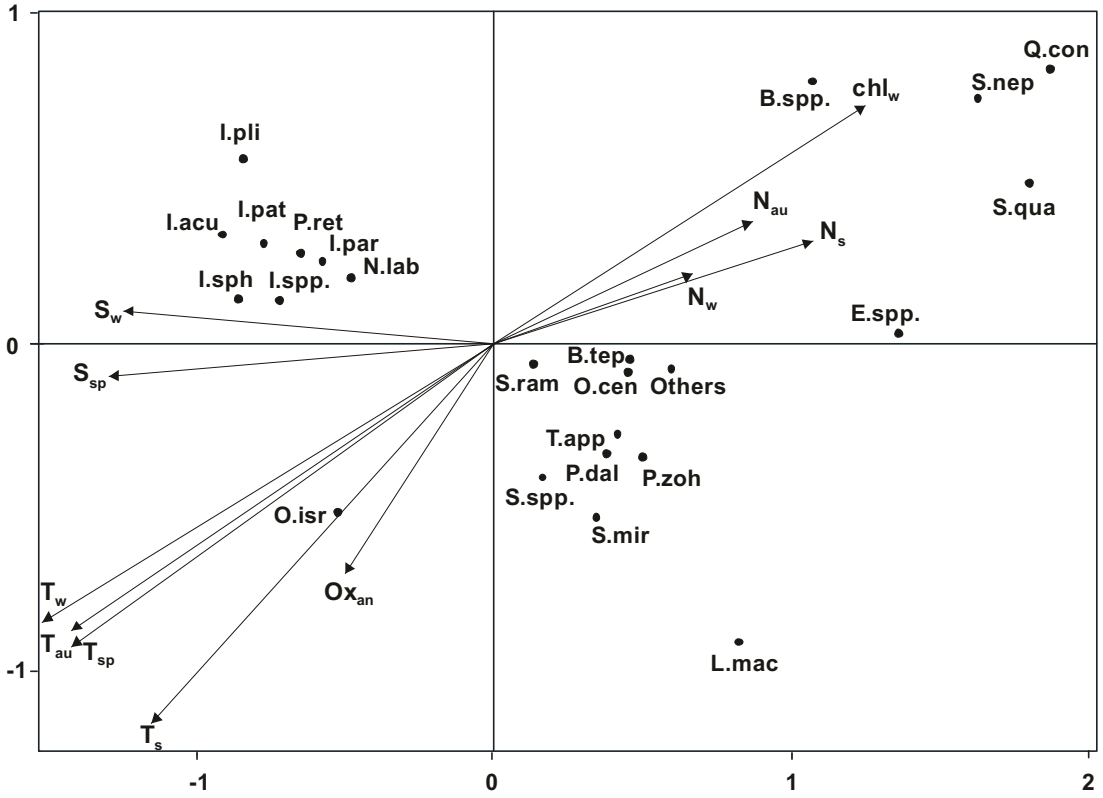

Figure 13. Ordination diagram of the CCA1 analysis. Arrows represent significant environmental parameters only. For abbreviations see Figure 12 and Table 2

Species of group 1 (*Impagidinium aculeatum, Impagidinium sphaericum, Impagidinium paradoxum, Impagidinium patulum, Impagidinium plicatum, Impagidinium* spp., *Operculodinium israelianum, Pyxidinopsis reticulata*, and *Nematosphaeropsis labyrinthus*) are ordinated at the positive side of the salinity,

bottom water oxygen, and temperature gradients and at the negative side of chlorophyll-*a* and nitrate gradients. *Operculodinium israelianum* occupies the most extreme position of this group. In contrast to the other species it is ordinated at the more positive side of the temperature gradients (Fig. 13).

Of the species forming group 2, *Selenopemphix nephroides*, *Echinidinium* spp., *Selenopemphix quanta, Quinquecuspis concreta*, and *Brigantedinium* spp. are ordinated at the positive side of the nitrate and chlorophyll-*a* gradients and at the negative side of temperature, bottom water oxygen, and salinity gradients (Fig. 13). *Lingulodinium machaerophorum* is ordinated at the negative side of the salinity axis, but at intermediate values of the other gradients (Fig. 13).

The other species are ordinated at the central part of the diagram. These species are *Bitectatodinium tepikiense, Operculodinium centrocarpum, Pentapharsodinium dalei, Polysphaeridium zoharyi, Spiniferites mirabilis, Spiniferites ramosus, Spiniferites* spp., *Trinovantedinium applanatum*, and the other rare taxa (*Ataxodinium choane, Bitectatodinium spongium, Impagidinium strialatum, Impagidinium velorum, Islandinium minutum, Protoperidinium americanum, Stelladinium stellatum, Tectatodinium pellitum, Tuberculodinium vancampoae, and Votadinium calvum)*. These species either have a random distribution or occur in low values in a few sites only (Fig. 13).

Table 3. Results of the Monte Carlo Permutation test of the CCA1 analysis. Marginal effects represent the amount of variance within the species data that is explained by that variable, uncorrected for covariance. Conditional effects represent the amount of variance explained by that particular variable after correction for covariance.

Marginal effect			**Conditional effect**		
Variable	**Lambda 1**	**Variable**	**LambdaA**	**P**	**F**
chl_s	0.21	T_w	0.23	0.005	10.46
T_w	0.21	chl_w	0.07	0.005	3.24
T_{sp}	0.19	T_{sp}	0.05	0.010	2.61
T_{au}	0.19	N_{au}	0.04	0.045	2.19
chl_{au}	0.19	N_s	0.05	0.025	2.83
chl_{sp}	0.18	Ox_{an}	0.03	0.070	1.79
S_{au}	0.18	S_{sp}	0.03	0.155	1.44
S_s	0.17	T_s	0.02	0.215	1.38
T_s	0.15	S_w	0.02	0.295	1.09
S_{sp}	0.15	N_w	0.02	0.235	1.17
S_w	0.14	T_{au}	0.02	0.285	1.11
chl_w	0.14	S_s	0.01	0.515	0.85
P_{au}	0.11	N_{sp}	0.01	0.935	0.40
N_s	0.10	chl_s	0.00	0.921	0.32
P_{sp}	0.09	chl_{au}	0.00	0.911	0.31
N_{au}	0.08	chl_{sp}	0.00	0.832	0.21
N_w	0.05	S_{au}	0.00	0.831	0.21
Ox_{an}	0.05	P_{au}	0.00	0.725	0.21
P_w	0.04	P_{sp}	0.00	0.721	0.10
P_s	0.04	P_w	0.00	0.667	0.10
N_{sp}	0.03	P_s	0.00	0.631	0.10

2.4.4 Geographic Distribution of Organic-Walled Dinoflagellate Cysts Based on the Absolute Abundance

2.4.4.1 Detrended Correspondence Analysis (DCA2)

The DCA2 reveals a gradient with a length of 2.243, indicating a unimodal distribution of the dinoflagellate cyst species. The first and second DCA2 axes represent 33.9% and 3% of the variance in the data set respectively. Higher axes explain less than 2% and are not taken into account.

The group of *Impagidinium aculeatum*, *Impagidinium sphaericum, Impagidinium paradoxum, Impagidinium* spp., *Impagidinium plicatum, Impagidinium patulum, Pyxidinopsis reticulata* and *Nematosphaeropsis labyrinthus* are ordinated together at the negative side of the first DCA2 axis where both the eastern and western Mediterranean Sea stations are ordinated as well (Figs. 14, 15). *Selenopemphix nephroides* and *Trinovantedinium applanatum* are ordinated at the positive side of the second axis corresponding to the position of western Mediterranean Sea stations (e.g. Alboran Sea) (Figs. 14, 15). *Selenopemphix quanta*, *Lingulodinium machaerophorum*, and *Echinidinium* spp. are ordinated at the most positive part of the first axis at a similar position of St. 572-2 that originates from the present day distal ends of waters influenced by the Nile River plume. (Figs. 14, 15). *Operculodinium israelianum* is ordinated at the positive side of the temperature gradient (Figs. 14, 15).

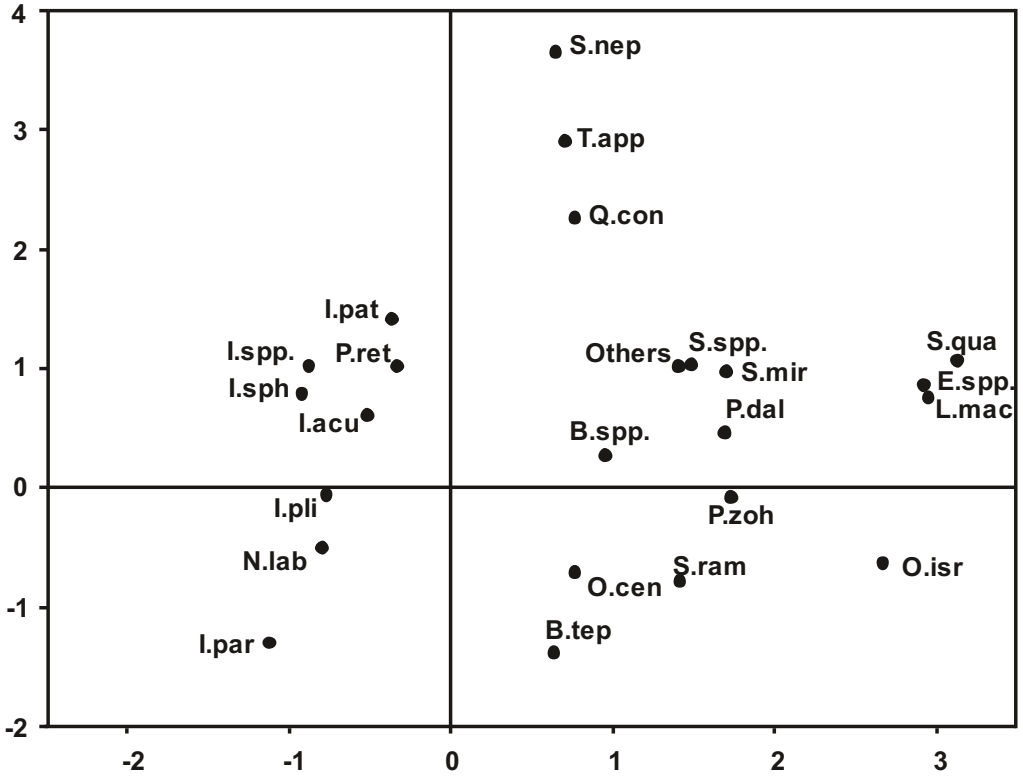

Figure 14. Ordination diagram of the DCA2 analysis showing the variation within the species distribution. For species abbreviations see Figure 12.

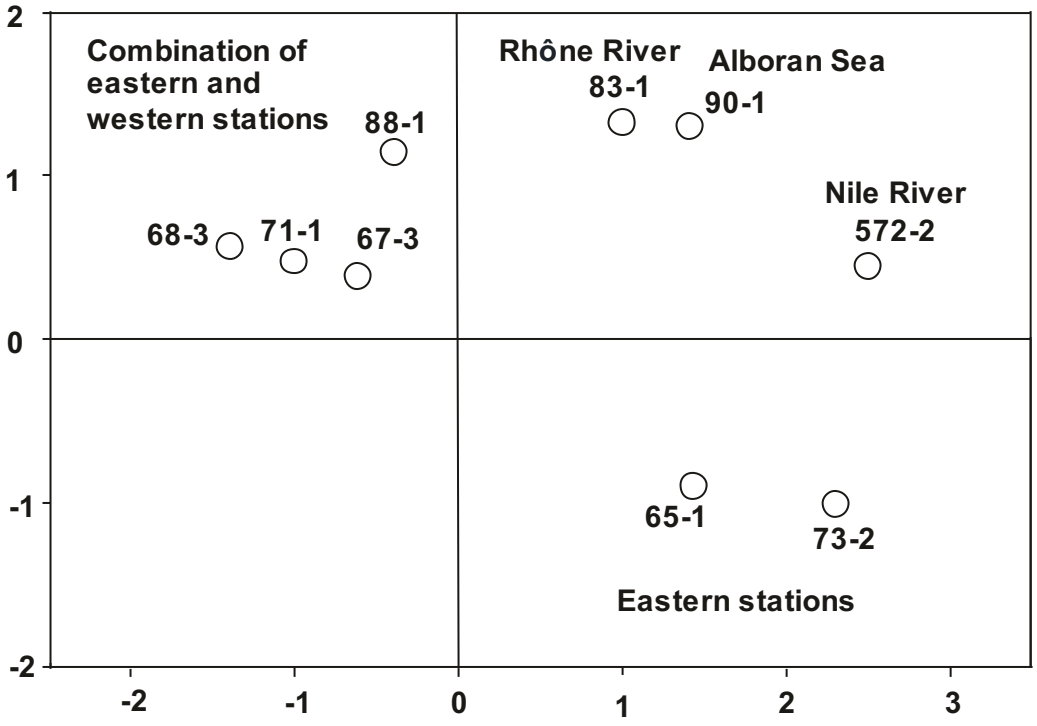

Figure 15. Ordination diagram of the DCA2 analysis showing the variation between sampling sites.

2.4.4.2 Canonical Correspondence Analysis (CCA2)

Monte Carlo Permutation test shows that the majority of the variation in cyst accumulation correlates to temperature variance in autumn (26%). The T_{sp}, N_{au}, T_w, N_w, S_w, and Ox_{an} vary significantly with the cyst accumulation and contribute to 14%, 15%, 11%, 7%, 5%, and 1% of the variation in cyst distribution respectively (Table 4).

Comparison with water column characteristics reveals that accumulation rates of *Impagidinium aculeatum, Impagidinium sphaericum, Impagidinium paradoxum, Impagidinium* spp., *Impagidinium plicatum,* and *Nematosphaeropsis labyrinthus* increase with increasing N in the upper water column and decreasing T and Ox_{an} in both the eastern and western Mediterranean Sea (Figs. 16, 17). *Operculodinium israelianum* has highest accumulation rates in the eastern Mediterranean Sea. It is ordinated at the positive side of salinity and temperature gradients, but at the negative side of the N gradient (Fig. 17).

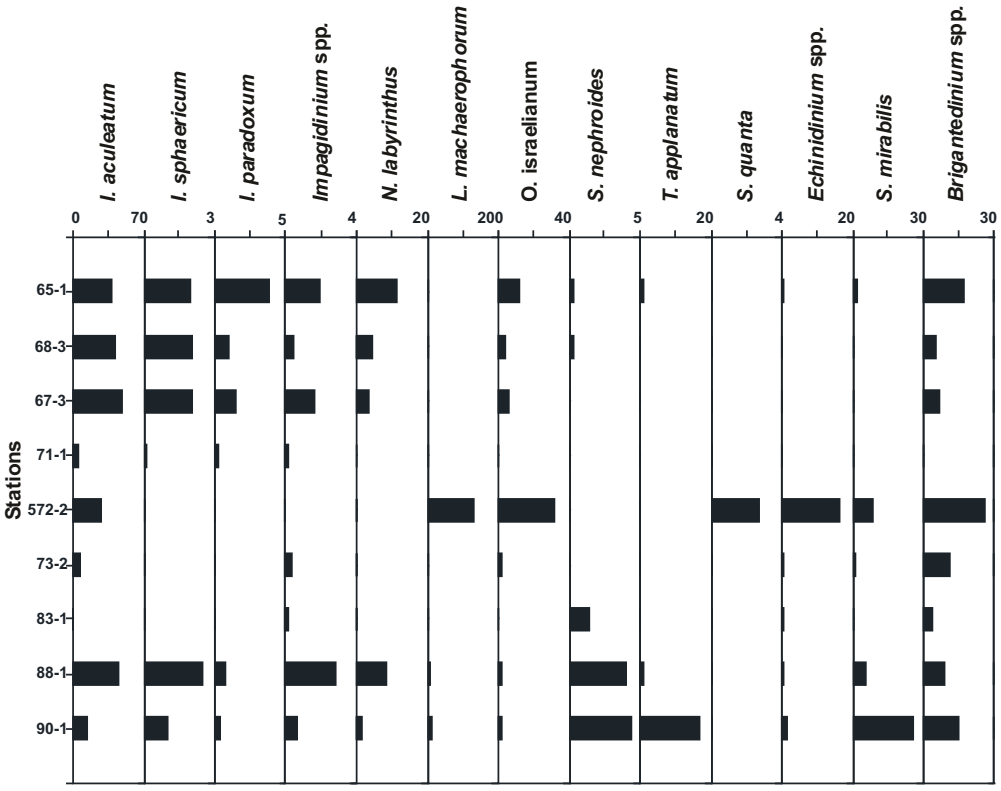

Figure 16. Accumulation rates of dinoflagellate taxa at the 9 sites where sedimentation rates are available based on ^{210}Pb and ^{14}C dating. The scale of each species starts with 0.

Selenopemphix nephroides and *Trinovantedinium applanatum* are ordinated at the positive side of the N gradient and at the negative side of salinity and temperature gradients (Fig. 17). *Selenopemphix nephroides* has high accumulation rates in the western high productive stations and at the most distal end of Rhône River (Fig. 16). *Trinovantedinium applanatum* is restricted to the high productive Alboran Sea (Fig. 16). All other cysts are ordinated in the centre of the diagram and do not have clear relation with the observed environmental parameters (Fig. 17).

Although *Spiniferites mirabilis* does not show a significant relation with different environmental parameters from the statistical analyses, it is worthy to note that this species shows a characteristic distribution with high relative and absolute abundances in the Alboran Sea (Figs. 10D, 16).

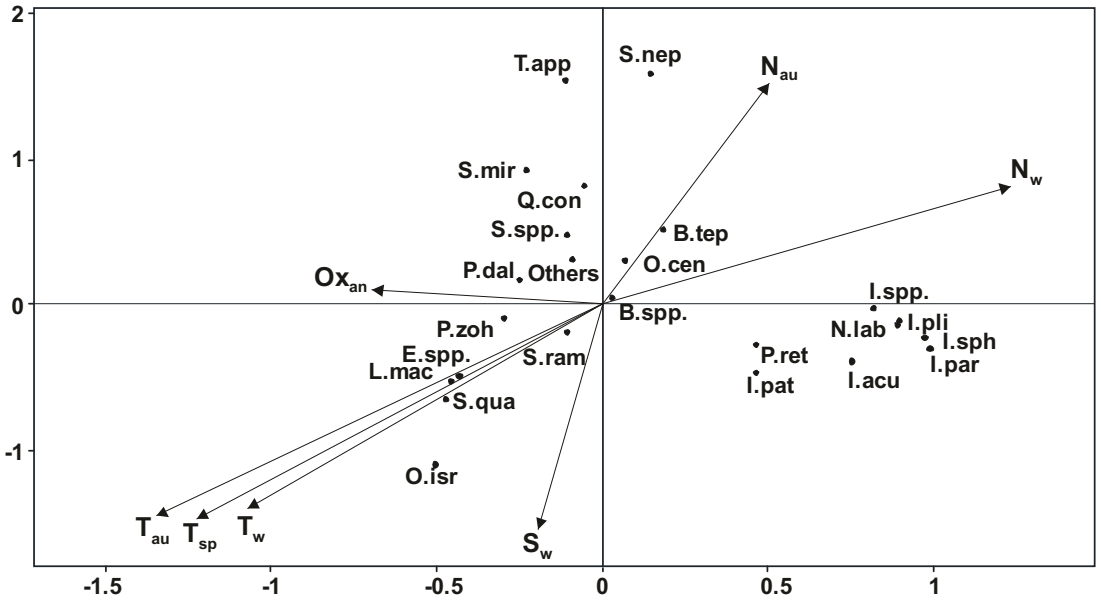

Figure 17. Results of the CCA2 analysis depicting the dinoflagellate cyst species in relation to significant environmental variables. For abbreviations see Figure 12 and Table 2.

Table 4. Results of the Monte Carlo Permutation tests of the CCA2 analysis.

Marginal effect				**Conditional effect**	
Variable	**Lambda 1**	**Variable**	**LambdaA**	**P**	**F**
T_{au}	0.26	T_{au}	0.26	0.02	3.44
T_s	0.25	T_{sp}	0.14	0.135	2.07
T_{sp}	0.25	N_{au}	0.15	0.03	3.02
T_w	0.22	T_w	0.11	0.06	3.07
P_{au}	0.21	N_w	0.07	0.045	3.20
S_{au}	0.20	S_w	0.05	0.075	1.67
N_{au}	0.20	Ox_{an}	0.01	0.185	1.10
S_s	0.20	T_s	0.00	0.231	0.91
S_w	0.19	P_{au}	0.00	0.295	0.87
S_{sp}	0.19	S_s	0.00	0.351	0.83
P_w	0.16	S_{au}	0.00	0.422	0.72
chl_s	0.15	P_w	0.00	0.453	0.61
N_w	0.15	S_{sp}	0.00	0.531	0.56
P_{sp}	0.15	P_{sp}	0.00	0.566	0.53
P_s	0.14	chl_s	0.00	0.677	0.42
chl_{sp}	0.14	N_s	0.00	0.652	0.33
N_s	0.13	P_s	0.00	0.640	0.21
chl_{au}	0.13	N_{sp}	0.00	0.732	0.20
chl_w	0.12	chl_{au}	0.00	0.821	0.11
N_{sp}	0.09	chl_w	0.00	0.933	0.10
Ox_{an}	0.08	chl_{sp}	0.00	0.910	0.10

Table 5. Raw count of the individual species of all analysed samples. Abbreviations: Wp: weight of processed sediment and Wi: weight of investigated sediment. For the other abbreviations of species see Table 6.

Station	Wp	Wi	A.cho*	B.spo*	B.tep	B.spp	E.spp	I.acu	I.par	I.pat	I.pli	I.sph	I.spp	I.str	I.vel
70-1	5.09	1.02	0	0	7	14	2	175	6	10	2	1	1	0	1
71-1	5.07	1.01	0	0	3	3	0	64	3	5	1	1	2	0	0
77A-1	5.57	0.56	2	0	1	17	10	65	3	5	0	1	0	0	0
77B-1	9.12	0.91	0	0	1	21	12	36	3	2	0	4	1	0	1
73-2	10.02	1.00	0	0	9	35	1	21	0	1	0	0	1	0	0
76-5	10.34	1.03	0	0	1	17	0	119	0	5	0	4	3	0	0
83-1	9.78	0.98	0	0	3	79	7	5	0	0	0	0	3	0	0
78-2	8.39	0.84	0	0	1	175	13	17	5	8	1	0	2	3	0
66-2	8.62	0.86	0	0	0	33	2	72	4	4	1	2	5	1	0
65-1	8.40	0.84	0	0	2	44	1	96	10	4	1	5	5	0	0
69-1	7.18	0.72	0	0	0	1	0	103	3	11	1	5	6	1	0
67-3	8.40	0.84	0	0	0	52	1	354	11	25	1	15	12	0	0
75-1	10.61	1.06	0	0	0	7	0	130	4	13	0	12	8	3	1
68-3	10.26	1.03	0	0	0	19	0	167	4	21	0	8	2	0	0
74-1	10.04	1.00	0	0	11	60	8	26	2	1	1	0	1	1	0
88-1	10.13	1.01	0	0	1	46	3	237	4	43	2	13	15	2	0
86-1	10.32	1.03	0	2	4	84	31	58	4	1	0	5	3	0	0
90-1	10.17	1.02	0	0	2	44	5	41	1	1	0	3	2	1	1
89-2	4.75	0.48	0	0	0	22	2	107	1	13	1	5	9	0	2
5845-1	9.96	1.99	0	0	1	3	3	26	1	2	0	0	2	0	1
5847-1	8.99	0.90	0	0	1	3	1	58	3	6	0	2	1	0	0
572-2	8.84	0.88	0	0	0	19	12	20	0	5	0	0	0	0	0
570	10.34	1.03	0	0	3	1	0	124	7	23	0	0	10	0	0
577-1	10.17	2.03	0	0	0	41	0	48	4	8	2	1	4	0	0
560-1	8.62	0.86	0	0	7	19	5	55	4	0	1	2	0	1	0
575-6	11.29	1.13	0	0	0	4	0	257	8	15	0	11	5	0	0
566-3	11.53	1.15	0	0	0	5	2	107	5	18	2	12	1	0	0
561	7.86	0.79	0	0	0	6	1	56	6	10	0	5	11	1	0
576-3	10.05	1.01	0	0	1	2	0	144	2	6	0	10	1	0	0
564-2	11.63	1.16	0	0	0	0	0	156	5	23	0	19	10	0	0
565-1	10.26	1.03	0	0	0	19	3	170	2	26	1	6	5	1	0
569-3	10.08	1.01	0	0	0	4	0	148	3	12	0	8	3	1	0
574-2	10.17	1.02	0	0	0	6	0	87	2	16	0	2	6	0	1
562-5	10.77	1.08	0	0	1	3	0	137	13	24	0	7	5	2	0

Table 5b.

Station	I.min*	L.mac	N.lab	O.cen	O.isr	P.dal	P.zoh	P.ame*	P.ret	Q.con	S.nep	S.qua	S.mir	S.ram	S.spp
70-1	0	3	3	9	7	1	2	0	8	0	0	0	0	1	7
71-1	0	0	3	2	3	1	2	0	1	0	0	0	0	0	15
77A-1	0	9	14	20	5	8	0	0	3	0	7	0	0	2	27
77B-1	1	8	8	14	3	21	8	1	0	0	9	1	4	2	35
73-2	0	9	0	46	5	24	7	0	0	0	0	0	3	4	33
76-5	0	5	1	3	10	4	1	0	0	0	0	0	0	0	9
83-1	0	4	2	18	4	8	3	0	0	2	28	2	3	3	15
78-2	0	7	7	16	0	6	3	0	1	0	26	1	4	6	32
66-2	0	2	14	38	25	11	3	0	7	0	1	0	7	7	21
65-1	0	1	29	76	31	13	7	0	0	0	1	0	5	9	64
69-1	0	0	5	4	8	3	1	0	4	0	0	0	2	2	5
67-3	0	13	25	10	39	2	0	0	7	0	1	0	4	9	47
75-1	0	8	11	18	43	6	2	0	3	0	0	0	2	3	48
68-3	0	2	17	3	15	0	1	0	7	0	1	0	1	0	8
74-1	0	34	5	52	3	70	23	0	1	2	8	1	14	16	97
88-1	0	25	45	90	8	37	12	0	4	0	21	0	31	9	285
86-1	0	37	31	65	5	52	24	0	2	1	59	3	13	9	152
90-1	0	38	4	60	4	19	4	0	4	0	13	0	76	3	255
89-2	0	7	12	40	5	22	5	0	3	0	3	0	8	1	104
5845-1	0	17	1	2	16	0	3	0	0	0	0	0	3	6	27
5847-1	0	31	1	6	49	1	1	0	3	0	0	0	5	2	67
572-2	0	94	0	14	23	10	5	0	1	0	0	2	6	4	43
570	0	19	0	4	32	1	1	0	0	0	0	0	3	2	20
577-1	0	3	1	0	1	0	1	0	2	0	0	0	1	3	7
560-1	0	4	6	26	8	7	3	0	3	0	3	0	14	6	39
575-6	0	12	14	4	20	1	2	0	4	0	0	0	4	1	30
566-3	0	5	14	11	54	0	2	0	3	0	0	0	4	13	34
561	0	15	5	48	9	15	3	0	1	0	15	0	14	3	192
576-3	0	11	32	4	19	2	3	0	4	0	0	0	2	18	52
564-2	0	3	6	1	10	0	0	0	4	0	0	0	2	0	15
565-1	0	1	14	14	37	6	4	0	2	0	2	0	9	2	45
569-3	0	19	11	1	20	2	2	0	7	0	0	0	7	6	61
574-2	0	22	14	2	25	4	1	0	0	0	1	0	9	2	88
562-5	0	3	1	6	26	5	1	0	2	0	0	0	11	0	65

Table 5c.

Station	S.ste*	T.pel	T.app	T.van	V.cal
70-1	0	0	1	0	0
71-1	0	0	0	0	0
77A-1	0	0	5	0	0
77B-1	0	0	14	0	0
73-2	0	0	0	0	1
76-5	0	0	0	1	0
83-1	0	0	1	0	0
78-2	0	0	8	0	0
66-2	0	0	0	0	0
65-1	0	0	2	0	0
69-1	0	1	0	0	0
67-3	0	0	1	0	0
75-1	0	0	0	0	0
68-3	0	1	1	0	0
74-1	0	0	3	0	0
88-1	0	0	6	2	0
86-1	1	0	27	0	2
90-1	0	0	50	0	0
89-2	0	0	2	0	0
5845-1	0	0	0	0	0
5847-1	0	0	0	0	0
572-2	0	0	0	0	0
570	0	0	0	0	0
577-1	0	0	0	0	0
560-1	0	0	0	0	0
575-6	0	0	0	0	0
566-3	0	0	1	0	0
561	0	0	0	0	0
576-3	0	0	0	0	0
564-2	0	0	0	0	0
565-1	0	0	0	0	0
569-3	0	0	0	1	0
574-2	0	0	0	0	0
562-5	0	2	1	0	0

Table 6. List of all the identified dinoflagellate cysts species with their abbreviations.

A.cho. *Ataxodinium choane*
B.spo. *Bitectatodinium spongium*
B.spp. *Brigantedinium* spp.
B.tep. *Bitectatodinium tepikiense*
E.spp. *Echinidinium* spp.
I.acu. *Impagidinium aculeatum*
I.par *Impagidinium paradoxum*
I.pat *Impagidinium patulum*
I.pli *Impagidinium plicatum*
I.sph *Impagidinium sphaericum*
I.spp. *Impagidinium* spp.
I.str. *Impagidinium strialatum*
I.vel. *Impagidinium velorum*
I.min. *Islandinium minutum*
L.mac. *Lingulodinium machaerophorum*
N.lab. *Nematosphaeropsis labyrinthus*
O.cen. *Operculodinium centrocarpum*
O.isr. *Operculodinium israelianum*
P.ame. *Protoperidinium americanum*
P.dal. *Pentapharsodinium dalei*
P.zoh. *Polysphaeridium zoharyi*
P.ret. *Pyxidinopsis reticulata*
Q.con. *Quinquecuspis concreta*
S.nep. *Selenopemphix nephroides*
S.qua. *Selenopemphix quanta*
S.mir. *Spiniferites mirabilis*

S.ram. *Spiniferites ramosus*
S.spp. *Spiniferites* spp.
S.ste. *Stelladinium stellatum*
T.app. *Trinovantedinium applanatum*
T.pel. *Tectatodinium pellitum*
T.van. Tuberculodinium vancampoae
V.cal. Votadinium calvum

2.5 Discussion

To obtain information on the ecological preferences of motile dinoflagellate species from the corresponding dinoflagellate cyst species distribution in the sediment, it is important to determine the possible effect of non-biological mechanisms on the dinoflagellate cysts that might have altered the assemblage.

2.5.1 Transport

Dinoflagellate cysts can be laterally displaced during or after settling to the sea floor. In the Mediterranean Sea, the effect of transport is difficult to estimate; since the general surface circulation transports upper waters eastwards down to about 200 m water depth whereas the underlying intermediate and deep waters flow westwards (Pinardi and Masetti, 2000). Bottom topography appears to be of great importance. The different basins of the Mediterranean Sea are separated by sills which prevent transportation from one basin to another once a cyst has sunk below sill depth, which is about 350 m for the Strait of Sicily (Pinardi and Masetti, 2000). This means that inter-basin long distance transport by currents is mainly limited to a west-east transfer within the eastward flowing Atlantic Water. The DCA1 statistical analysis clearly separates eastern offshore stations and western productive stations (Fig. 11). Consequently we assume that large scale transport/exchange between two main Mediterranean sub-basins does not affect the sedimentary cyst distribution. It is well known that marine biogenic particles produced in the upper water masses can be laterally transported on their way to the ocean floor. In the last decades, it has become clear that most marine organic matter does not sink as individual particles but settle as (phyto) plankton derived aggregates, faecal pellets or marine snow (Smayda, 1970; Mudie, 1996; Turner, 2002; Fischer et al., 2009). Recent sediment trap studies have revealed that the sinking velocities of dinoflagellate cysts are fast (at least 274 m/d) corresponding to downward transport in the form of phytoplankton aggregates or faecal pellets (e.g. Zonneveld and Brummer, 2000; Turner, 2002;

Zonneveld et al., in press a). However, although this suggests minimal risk of lateral transport during the sinking processes, these studies are based on the conditions in high productivity regions. Since no information is available so far for the low productivity Mediterranean Sea, we have no information if the lateral transport during initial settling has affected the cyst distribution in our research area.

Marine biogenic particles can also be relocated for long distances after their initial deposition within bottom-boundary nepheloid layers or with large scale sediment transport processes such as turbidity currents which latter are a common phenomenon in the Mediterranean Sea (e.g. Stanley and Maldonado, 1979; Mulder et al., 2001; Khripounoff et al., 2009 and references therein). If lateral transport would strongly influence the cyst distribution pattern in our research area, we would expect to find a gradual change in associations and concentrations from the shelf to the deeper sites or enhanced cyst accumulation rates in the turbidity depo-centers. In our study we do not see such a gradual change in associations from the shelf to deeper sites but observe that upper ocean conditions are reflected in the cyst-associations. For instance, the bands of high productivity in upper waters related to the Rhône, Nile and Po-River plumes are reflected by a typical cyst association in the underlying sediments with no relationship to water depth. Unfortunately the limited amount of sites from which we have detailed information about the local sedimentation rates hampers us to determine if cyst accumulation rates are enhanced in sediment depo-centers. Although we have no indication that sediment rearrangement post-depositionally has disturbed our cyst distribution patterns, we can not exclude it. Nevertheless, both visual examination of the dataset as well as the results of the statistical analysis reveal that upper ocean oceanographic conditions are reflected in our sedimentary cyst association. We therefore assume that the potential post depositional lateral displacement of sediments did not significantly disturb the initial signal.

2.5.2 Preservation

Recent studies have shown that oxygenated bottom waters can cause species-selective post-depositional degradation, altering dinoflagellate cyst association (Zonneveld et al., 1997; 2007; 2008; Zonneveld, Versteegh et al., 2001; Bockelmann et al., 2007; Kodrans-Nsiah et al., 2008). We observe in this study, a significant

correlation between the relative cyst abundances of individual species and oxygen concentrations in bottom waters which is elucidated by CCA1 analysis (Fig. 13). Cysts in the group 1 (such as *Impagidinium* species, *Operculodinium israelianum*, and *Nematosphaeropsis labyrinthus*) have their highest relative abundances in deep eastern Mediterranean sites where bottom waters are characterized by relatively high oxygen concentrations. As known from previous studies, this group is particularly resistant to pre- or post-depositional aerobic degradation (Kodrans-Nsiah et al., 2008; Zonneveld et al., 2007; 2008). The high relative abundances of these species in these sites might therefore be the result of species-selective degradation rather than ecological affinity of their motiles. All the cysts in the group 2 with exception of *Lingulodinium machaerophorum* are known to be vulnerable to aerobic degradation. They are found in western Mediterranean or at the distal end of river plumes where bottom water has relatively low oxygen concentration. As a result we cannot exclude oxygen as a factor that could have altered our cyst associations post-depositionally and our data should be interpreted taking into consideration both the ecological and degradation signals.

2.5.3 Dinoflagellate Cysts Distribution Based on Relative and Absolute Abundance in Relation to Environmental Parameters

Both visual observation and the statistical analysis show that the dinoflagellate cyst distribution is related to combination of different environmental parameters.

Based on the relative abundance distribution patterns we determine two cyst associations that can be related to different oceanographic regimes; (1) Offshore eastern Mediterranean (2) Western Mediterranean Sea, Strait of Sicily/NW Ionian Sea, and/or distal end of river plumes.

Group 1: The offshore eastern Mediterranean association consists of the species *Impagidinium aculeatum, Impagidinium sphaericum, Impagidinium patulum, Impagidinium paradoxum, Impagidinium* spp., *Impagidinium plicatum, Pyxidinopsis reticulata*, *Nematosphaeropsis labyrinthus,* and *Operculodinium israelianum*. Species of this group have high relative abundances in areas characterized by warm, high salinity surface waters with low chlorophyll-*a* concentration, and bottom water that have relatively high oxygen concentration (Fig. 13). They show increasing

accumulation rates with increasing nitrate concentrations but with decreasing surface water temperatures (Fig. 17).

The dominance of these species in oligotrophic regions with high bottom water oxygen concentrations are in accordance with the previous studies in the Mediterranean (Rubino et al., 2000; Mudie et al., 2004; Sangiorgi et al., 2005; Giannakourou et al., 2005; Marret et al., 2008; Zonneveld et al., in press a) . Studies from the Aegean Sea and Black Sea show that the autotrophic gonyaulacoids *Operculodinium israelianum, Polysphaeridium zoharyi, Impagidinium aculeatum, Impagidinium patulum, Impagidinium sphaericum,* and *Impagidinium strialatum* form an association characteristic for the hypersaline Aegean Mediterranean water, whereas heterotrophic protoperidinioids are observed in relative high abundances in areas influenced by low-salinity Marmara-Black Sea water.

The information about the ecological significance of dinoflagellate cysts based on their accumulation rates is extremely sparse (Montresor et al., 1998; Zonneveld and Brummer, 2000; Mudie et al., 2001; Harland et al., 2004; Morquecho and Lechuga-Devéze, 2004; Susek et al., 2005; Dale, 2009; Zonneveld et al., in press b). Changes in cyst proportion are often interpreted to reflect changes in cyst production. This can lead to completely wrong conclusions when the data suffer from so called, closed-sum effects and / or are overprinted by degradation (Zonneveld et al., in press a). All species of group 1 are known to be extremely resistant against aerobic degradation. As result their accumulation rates are likely to reflect their initial production and as such can be used to obtain information about the ecology of the species.

Surface-water temperature is selected by CCA1 and CCA2 as the most important environmental factor influencing the Mediterranean cyst distribution. All species of group 1 are known to be phototrophic. Higher temperature might have a direct positive effect on the metabolic rate of these species, and hence dinoflagellate growth in general. It also enhances the vertical stability of the water column, which most phototrophic dinoflagellate taxa tend to favour (Taylor and Pollingher, 1987; Kjoeret et al., 2000; Godhe and McQuoid, 2003).

Focusing on individual taxa, we find that temperature relates positively to both the relative abundance and the accumulation rate of *Operculodinium israelianum* (Figs.

13, 17). *Operculodinium israelianum* is observed only in the eastern warm Mediterranean waters. Based on its global relative abundance pattern, Marret and Zonneveld (2003) suggested that *Operculodinium israelianum* might be a typical warm water species. Other studies confirm such a positive relation between temperature and the absolute or relative abundance of *Operculodinium israelianum* (Pospelova et al., 2004; Zonneveld et al., in press a). Since we observe a positive relationship between surface temperature and accumulation rate of this species (Fig. 17), we assume that temperature indeed might be a causal factor influencing its distribution.

Although the relative abundances of the other species of this group are positively related to upper ocean temperatures, their cyst accumulation is not. Consequently we assume that temperature does not form a causal factor influencing the distribution pattern of these species in our research area.

The second and the third most important variables emerging in the CCA1 analysis are chlorophyll-*a* and nitrate concentration. Previous studies generally defined species from the genus *Impagidinium* as characteristic for oligotrophic and oceanic conditions (e.g. Wall et al., 1977; Edwards and Andrle, 1992; Devillers and de Vernal, 2000; Persson et al., 2000). However, *Impagidinium patulum* is the only species from the species classified in group 1, which is characteristically found in high relative abundances in low productivity regions (e.g. Dale, 1986; Zonneveld, 1997; Dale, 1996; de Vernal et al., 1998; Devillers and de Vernal, 2000; Dale et al., 2002; Marret and Zonneveld, 2003; Marret et al., 2004; Matthiessen et al., 2005; Bockelmann et al., 2007; Esper and Zonneveld, 2007; Holzwarth et al., 2007; Radi et al., 2007; Pospelova et al., 2008). The other species in this group can frequently be found in high relative abundances in eutrophic areas as well (Marret and Zonneveld, 2003).

The accumulation rates of all species in the group 1, except *Impagidinium patulum, Operculodinium israelianum*, and *Pyxidinopsis reticulata*, show in our study a positive relationship with nitrate concentration and are found in both the eastern and western Mediterranean. A similar relationship is observed in the Arabian Sea where *Impagidinium aculeatum* shows enhanced cyst production during the upwelling season (Zonneveld, 1997). Zonneveld et al. (in press b) found that species of

Impagidinium and *Nematosphaeropsis* show increased cyst production in relationship to increased nutrient and trace-element concentrations in the upper waters of NW Africa in a long-term sediment trap study. We therefore assume that nitrate concentration might form a causal factor influencing the cyst accumulation of the *Impagidinium* and *Nematosphaeropsis*. Dale (1992) reports that the cyst production of *Impagidinium* species is often extremely low as registered by sediment trap studies in both eutrophic and oligotrophic regions. Consequently, the relative abundance data of this group is likely to be overprinted by closed sum and preservation effects.

Group 2: Western Mediterranean, Strait of Sicily/NW Ionian Sea stations, and/or distal river outlets association.

With exception of *Lingulodinium machaerophorum* the relative abundances of this group, containing *Selenopemphix nephroides, Echinidinium* spp*., Selenopemphix quanta, Quinquecuspis concreta, Brigantedinium* spp. increase with increasing productivity, but with decreasing sea-surface salinity, temperature and bottom water oxygen concentration.

Concerning the temperature factor, we find that both the absolute and relative abundances of *Selenopemphix nephroides* and absolute abundance of *Trinovantedinium applanatum* are associated with cooler environments in the research area (Figs. 13, 17). Some previous studies are in agreement with this result (e.g. Pospelova et al., 2006; 2008). However, when considering their global distribution they are most abundant in sub- to tropical regions, whereas they have low abundances in colder regions (e.g. Marret and Zonneveld, 2003). We therefore assume that temperature is not a causal factor influencing their distribution in this study.

The high ranking of productivity in the CCA1 analysis reflects the importance of the productivity especially for some of the heterotrophic dinoflagellate, such as *Selenopemphix nephroides, Echinidinium* spp*., Selenopemphix quanta, Quinquecuspis concreta*, and *Brigantedinium* spp. (Fig. 13). Increasing productivity might increase growth of the food source of heterotrophic dinoflagellates. It is well known that heterotrophic species can feed on numerous food sources such as

diatoms, flagellates, bacteria, ciliates, copepod eggs, haptophytes, nauplius larves, dinoflagellates, and organic debris (e.g. Jacobson and Anderson, 1986; Menden-Deuer et al., 2005; Gribble et al., 2007). Cyst production of heterotrophic dinoflagellates is often closely coupled to the timing of maximal food abundance because of their more rapid reproduction rates (Archer et al., 1996; Kjoeret et al., 2000; Zonneveld, et al., in press b). It is therefore not surprising to find a positive relationship between peridinoid groups and the chlorophyll-*a* vector. However, only the accumulation rates of *Selenopemphix nephroides* and *Trinovantedinium applanatum* increase in relationship with increasing nutrient concentrations in the upper waters (Fig. 17). No relationship between accumulation rates of *Echinidinium* spp*., Brigantedinium* spp., *Selenopemphix quanta,* and *Quinquecuspis concreta* and environmental parameters in surface waters can be observed. As mentioned previously these species especially *Echinidinium* spp. and *Brigantedinium* spp. are extremely sensitive for post depositional degradation. As in our research area bottom water oxygen concentrations strongly co-vary with nutrient and chlorophyll-*a* concentrations, it is therefore likely that the relationship of the dinoflagellate cyst accumulation rates with these environmental factors is disturbed by post depositional degradation processes. For *Selenopemphix nephroides* and *Trinovantedinium applanatum* the positive relationship with enhanced nutrient concentrations might be so strong that post depositional diagenetic overprint did not completely destroy the initial ecological signal.

Salinity is ranked as the least influential environmental parameter. In our study the coincidence between low salinity and high productivity regions could not allow us to clearly differentiate between salinity and the productivity signal. We assume that the combination of both factors influenced the cyst association. Previous studies in regions of river and upwelling systems where bottom waters contain low oxygen concentrations indicate that cyst assemblages in these regions are often strongly dominated by heterotrophic taxa (Wall et al., 1977; Marret, 1994; Dale, 1996; Zonneveld, 1997; Zonneveld, Hoek et al., 2001; Dale et al., 2002; Marret and Zonneveld, 2003; Sprangers et al., 2004; Susek et al., 2005; Holzwarth et al., 2007; Pospelova et al., 2008). This holds in particular regions such as in the Po-River, the Marmara Sea, and the south-eastern Mediterranean Egyptian coast and shelf

(Rubino et al., 2000; Mudie et al., 2004; Sangiorgi et al., 2005; Kholeif, 2007; Zonneveld et al., in press a).

We record high relative abundances and accumulation rates of *Selenopemphix nephroides* in the high productivity regions of the western Mediterranean Sea, the Strait of Sicily/NW Ionian Sea, and at the distal ends of the river plumes. The global distribution of this species is not restricted to the distal river plume areas, but is generally observed in high relative and absolute abundances at sites with high nutrient trace element concentrations in upper waters (e.g. Marret and Zonneveld, 2003; Dale, 2009; Zonneveld et al., in press a). In the Aegean-Black Sea corridor, the distribution of *Selenopemphix nephroides* indicates that this species even tends to avoid very low surface water salinity concentrations (Mudie et al, 2004). As a result, we consider this species in our research area as useful to reconstruct past changes in trophic level rather than for salinity changes.

In our study *Lingulodinium machaerophorum* is recorded in high relative and absolute abundances in stations at the distal end of the Nile influence (Figs. 10C, 16). Previous studies confirmed that *Lingulodinium machaerophorum* is often observed in high and sometimes overwhelming amounts present in sediments from river plumes (Lewis, 1988; Dale and Fjellsa, 1994; Lewis and Hallett, 1997; Dale et al., 1999; de Vernal et al., 2001; Dale et al., 2002; Pospelova et al., 2004; Wang et al., 2004; Pospelova et al., 2005; Dale, 2009; Zonneveld et al., in press a). In the north-western Adriatic Sea the positive shift in the relative abundance of *Lingulodinium machaerophorum* suggests an increase in eutrophication (Sangiorgi and Donders, 2004). In addition, pilot studies carried out in vicinity of the Nile Delta register high abundances of *Lingulodinium machaerophorum* close to the Nile outlet (Marret et al., 2008; Kholeif, 2009). This seems to be in contrast with the findings from Kholeif and Mudie (2009) who did not record *Lingulodinium machaerophorum* cysts in post-sapropelic sediments in a core ca 50 km north of the Nile Delta. On first sight, it can be assumed that previous to the construction of the Aswan Dam the outflow of the Nile must have been so much larger, that discharge waters reached a more northern position than today (Nixon, 2003). However, the Nile outflow waters are pressed against the south-eastern margin of the Levantine Basin by coriolis force and the upper water currents (Fig. 5). Since there are no indications that upper water

circulation has changed in the region in the Late Holocene, it can be expected that this situation has occurred throughout the late Holocene. Enhanced outflow compared to today would result in a prolongation of the discharge plume along the coast but not on more basin-ward extension of the plume. This suggest that *Lingulodinium machaerophorum* might form a suitable marker to trace past variations in river discharge, notably from the Nile. This is strengthened by the fact that *Lingulodinium machaerophorum* is found to be moderate resistant to resistant against aerobic degradation, minimizing the risk that diagenetic overprint destroys the initial signal post-depositionally (Zonneveld et al., 2008).

All other species are ordinated in the central part of the CCA1 diagrams (*Bitectatodinium tepikiense*, *Operculodinium centrocarpum*, *Pentapharsodinium dalei*, *Polysphaeridium zoharyi*, *Spiniferites mirabilis*, *Spiniferites ramosus*, *Spiniferites* spp., and *Trinovantedinium applanatum)* (Fig. 13). This indicates that their distribution is either not influenced by the observed environmental parameters or their highest cyst abundances occur in sites where the environmental parameters have intermediate values.

Of these species *Bitectatodinium tepikiense* was previously described as characteristic for cold/temperate regions (Marret and Zonneveld, 2003), and is generally used as a cold water dinoflagellate cyst indicator (Shaozhi et al., 2002). In the Mediterranean Sea *Bitectatodinium tepikiense* is not characteristically present in the colder regions of the basin. Several studies associate *Spiniferites ramosus* with warmer water, while *Pentapharsodinium dalei* can generally be related to cooler environment (Pospelova et al., 2008: Bouimetarhan et al., 2009). Our study does not confirm these observations.

Spiniferites mirabilis is generally found to have high relative abundances in sites where warmer surface water conditions occur (e.g. Pospelova et al., 2008; Bouimetarhan et al., 2009). Here we find only a very slight positive relationship between temperature and the relative abundances but no relationship when accumulation rates are considered. We therefore assume that temperature is not a strong steering factor in this study. We observe high relative abundances and accumulation rates of this species in the Alboran Sea. Since the Alboran Sea is a transition zone between the Mediterranean Sea and Atlantic Ocean, this species

might form an indicator for the boundary of these two waters in the study area. This might be supported by the fact that *Spiniferites mirabilis* is often observed in high relative abundances in near frontal systems when considering its global distribution (Marret and Zonneveld, 2003). Morzadec-Kerfourn (1988; 1992; 2005) considers *Spiniferites bentorii* to be indicative for sea-level change in the Western Mediterranean area. Our results do not show a depth dependence of this species, therefore we can not confirm this statement.

Operculodinium centrocarpum is often characterized as a cosmopolitan species, which is commonly found in coastal waters, but can also be documented in increased relative abundances in unstable waters (e.g. Dale, 1992). This might explain why in this study *Operculodinium centrocarpum* is found everywhere in the Mediterranean Sea and does not show a significant relation with any observed environmental parameter.

The current study documents that the distribution of dinoflagellate cysts in surface sediment samples of the Mediterranean Sea reflects upper and bottom water environmental conditions, notably gradients in upper ocean temperature, the trophic state of the upper waters, and bottom water oxygen concentrations. Whereas changes in abundances of oxidative sensitive species might provide information about the redox state of the bottom waters, the accumulation rates of oxidative resistant species appear to be positively related to the trophic state of the surface waters as reflected by the upper water nitrate concentration. Since human induced eutrophication effects often result in anoxic bottom water conditions, our findings strongly support dinoflagellate cysts to be extremely suitable proxies to study the effects of human eutrophication in the past Mediterranean Sea region.

2.6 Conclusions

The dinoflagellate cyst associations of 34 surface sediment samples from both the western and eastern Mediterranean Sea can be related to both upper and bottom water characteristics.

No indication for lateral relocation of cysts during the settling process or after deposition can be observed. The strong relationship between bottom-water oxygen

concentration and the cyst distribution indicates that the cyst associations might have been affected by post-depositional species selective degradation.

Based on the relative abundance data, two associations can be distinguished that are characteristic for the major oceanographic settings in the region.

Association 1 is characteristic for the offshore eastern Mediterranean Sea and consists of the species *Impagidinium aculeatum, Impagidinium sphaericum, Impagidinium patulum, Impagidinium paradoxum, Impagidinium* spp., *Impagidinium plicatum, Pyxidinopsis reticulata, Nematosphaeropsis labyrinthus* and *Operculodinium israelianum*. Species of this group have high relative abundances in areas characterized by warm, high salinity, surface waters with low chlorophyll-*a* concentration, and bottom water that have relatively high oxygen concentration. Temperature is positively related to the cyst accumulation of *Operculodinium israelianum*, but does not form a causal factor influencing the distribution of the other species in this group. The accumulation rates of species of the genus *Impagidinium* and *Nematosphaeropsis labyrinthus* increase with increasing N concentrations in upper waters.

Association 2 is characterised by species having their highest relative abundances in sediments from the western Mediterranean Sea, Strait of Sicily/NW Ionian Sea and at the distal end of river plumes. At these sites, surface waters are characterized by high primary productivity associated with low SST and SSS, whereas bottom water oxygen concentrations are relatively low. The cyst association consists of *Selenopemphix nephroides, Echinidinium* spp., *Selenopemphix quanta, Quinquecuspis concreta, Brigantedinium* spp. and *Lingulodinium machaerophorum*. Relative abundances and accumulation rates of *Selenopemphix nephroides* are positively related to upper water chlorophyll-*a* and nitrate concentrations indicating that this species might form valuable eutrophication indicator in the study region. *Lingulodinium machaerophorum* is found in high abundances in samples located at the distal ends of river discharge plumes especially in the Nile plume. It might therefore form a suitable marker to trace past variations in river discharge, notably from the Nile.

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Chapter 3

Spatio-temporal distribution of neritic benthic foraminifera in relation to anthropogenic activities in Abu-Qir Bay, Alexandria, Egypt

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Abstract

The present study deals with the foraminiferal distribution in recent bottom sediments of Abu-Qir Bay located along the Mediterranean coast of Alexandria, Egypt. Abu-Qir Bay receives substantial amounts of heavy metals from the surrounding industrial area, agricultural and domestic effluents, making it one of the most polluted areas along the Mediterranean coast of Alexandria. The environmental characterisation, carried out using the study of benthic foraminifera, was integrated with geochemical, sedimentological, and water environmental parameters because these organisms are recognised as excellent tools for pollution assessment in marine environments. A total of 18 sediment samples were collected at 2 sampling periods in 2005. Cluster analysis (CA) and Canonical Correspondances Analysis (CCA) reveal a distinctive boundary separating two groups; the off shore less polluted stations and the onshore polluted stations. The first group is characterised by sediment rich with total organic carbon, high silt and clay content, and high bottom water temperature, but low dissolved oxygen concentrations, salinity, transparency, and pH and dominated mainly by *Ammonia tepida, Quinqueloculina lata* and *Porosononion* spp. These species are considered as pollution opportunists. The second group is characterised by coarser sediment with high bottom oxygen concentration, and transparent, saline and cold water, and is dominated mainly by sensitive species such as *Quinqueloculina vulgaris, Elphidium* spp., *Asterigerinata mamilla, Rosalina macropora, Ammonia beccarii, Triloculina trigonula, Peneroplis pertusus,* and *Quinqueloculina* spp. Heavy metals pollution has a deleterious effect on benthic foraminifera e.g., reduced population diversity, increase in dominance, and the frequent presence of deformed tests.

3.1 Introduction

Benthic foraminifera are used as environmental bioindicators especially in polluted environments where their sensitivity to pollutants may be expressed by a modification of their test structure or by a change in the composition of the assemblage. Pollution impact studies on benthic foraminifera have been initiated by Resig (1960) and Watkins (1961). Subsequent studies have often focused on areas exposed to direct pollution sources, such as industrial, sewage or agricultural wastes. Foraminifera respond to anthropogenic changes (organic and inorganic causes) and

during the last years several workers have tried to relate foraminiferal distribution patterns to different kinds of pollution. Most of these studies have focused on the effect of sewage outfalls on the foraminiferal assemblages (Bandy et al., 1964a, 1964b; Seiglie, 1968, 1975; Schafer, 1973; Bates and Spencer, 1979). Few investigations concentrate on faunal modifications caused by paper and pulp mills (Schafer, 1973; Buckley et al., 1974; Alve and Nagy, 1986; Nagy and Alve, 1987). Some studies have also taken into account thermal and various kinds of chemical pollution (Schafer, 1970, 1973; Buckley et al., 1974; Seiglie, 1975) and heavy metal pollution (Yanko et al., 1998; Samir, 2000; Samir and El-Din, 2001; Debenay et al., 2001; Armynot du Chatelet, 2003; Armynot du Chatelet et al., 2004; Bergin et al., 2006; Le Cadre and Debenay, 2006; Romano et al., 2008; Martins et al., 2010). These studies showed a significant relation between foraminifera species and heavy metal pollution and provided evidence for pollution-tolerant species, such as *Ammonia tepida* (Samir, 2000; Samir and El-Din, 2001; Bergin et al., 2006), *Haynesina germanica* (Ehrenberg) (Armynot du Chatelet et al., 2004), *H. germanica* and *Quinqueloculina parvula* (Romano et al., 2008). These studies suggested also that heavy metals can penetrate the foraminiferal cell together with food (e.g., algae, bacteria) and then affect the foraminiferal cytoskeleton, which defines the shape of the organism, giving place to test deformations (Le Cadre and Debenay, 2006).

Abu-Qir Bay was considered as one of the most profitable fishing areas before it was polluted by the disposal of sewage and industrial effluents. It receives different types of pollutants; industrial, sewage, and agricultural. Abu-Qir Bay receives industrial wastewaters from more than 36 different factories of food processing and canning, paper industry, fertilizer industry, and textile manufacturing (Said et al., 1995). Consequently Abu-Qir Bay is particularly suitable for the pollution characterisation and determination of environmental stress using benthic foraminifera.

In comparison to the previous studies regarding the chemistry of sediments, water, and sedimentology in Abu-Qir Bay (El Nabawi et al., 1987; Badr, 1993; Frihy et al., 1994; Said et al., 1995; Abdel-Moati, 1997; Stanley et al., 2004; Frihy et al., 2008; Kamal, 2008), no attempts have been made to elucidate the effect of pollution on benthic foraminiferal communities in this area. Concerning the study of benthic foraminifera from the beaches of Alexandria, little concern has been paid to this

issue (e.g., Said and Kamel, 1954, 1957; El-Halaby, 1975; Abu El-Enien, 1979; El-Menhawey, 1998) and few attempts have been made to show the pollution effect on benthic foraminifera (Samir, 2000; Samir and EL-Din, 2001). Samir (2000) showed that benthic foraminifera are more sensitive to industrial wastes containing heavy metals as in Manzalah Lagoon than agricultural wastes as in Idku Lagoon. He concluded also that *A. tepida* is more resistant to pollution than *Ammonia parkinsoniana*, morphological abnormalities of the foraminiferal tests depend upon the nature of the pollutant, and benthic foraminifera are less tolerant to pollution than ostracods and molluscs. Samir and EL-Din (2001) indicated that increasing pollution results in low species diversity and population density, associated with an increase in tolerant or opportunistic species. They strongly suggested that heavy metals are responsible for the abnormalities in foraminiferal tests as indicated from X-ray microanalysis. So the heavy metal aspect will be addressed in detail in the present study. Despite the complex spatial and temporal patterns of environmental characteristics, most of foraminiferal studies focus on their spatial distribution, only few studies concern the seasonal changes in living foraminiferal assemblages (Murray and Alve, 2000; Debenay et al., 2006; Duchemin et al., 2008).

The main goal of this study is to investigate the distribution, abundance and diversity of benthic foraminifera, to: 1) examine the quantitative distributions of deformed foraminifera, and 2) determine the pollution tolerant and pollution sensetive bio-indicators that can be used to characterize the heavy metal pollution in Abu-Qir Bay, taking into consideration the effect of other environmental parameters. The sampling stations were chosen according to the different pollution level. Moreover the seasonal aspect is considered.

3.2 Study area

Abu-Qir, the most industrialized district in Alexandria, is extending between the town of Abu-Qir and the Rosetta mouth of the River Nile. It is situated between El-Maamoura in the west and the Rosetta mouth of the Nile in the east. It lies between longitudes 30˚5' and 30˚22'E and latitudes 31˚16' and 31˚28'N (Fig. 1). It is a semicircular tideless basin with an area of about 500 km^2 and average depth of ≈2 m. The bay receives considerable amounts of waste waters through 2 main sources: (1)

Industrial wastes through El-Tabia pumping station. These wastes are coming from about 36 factories extending from Kafr El-Dawar to Alexandria in a cultivated area. Most of these factories were constructed since the middle 1950s. (2) Brackish water polluted by agricultural runoff of Lake Edku discharged through Boughaz El-Maadiya, which is a shallow narrow channel of about 2 m depth, 20 m width and 100 m long. This run off usually contains different types and quantities of pesticides which differ according to season and depending upon the types and intensities of agriculture near the coast. The annual average of the drainage water discharged into the lake is about $1x10^9$ m^3 (EL-Sayed et al., 1993). Average concentrations of nutrients and metals in water discharged from Lake Edku are given in Table 1.

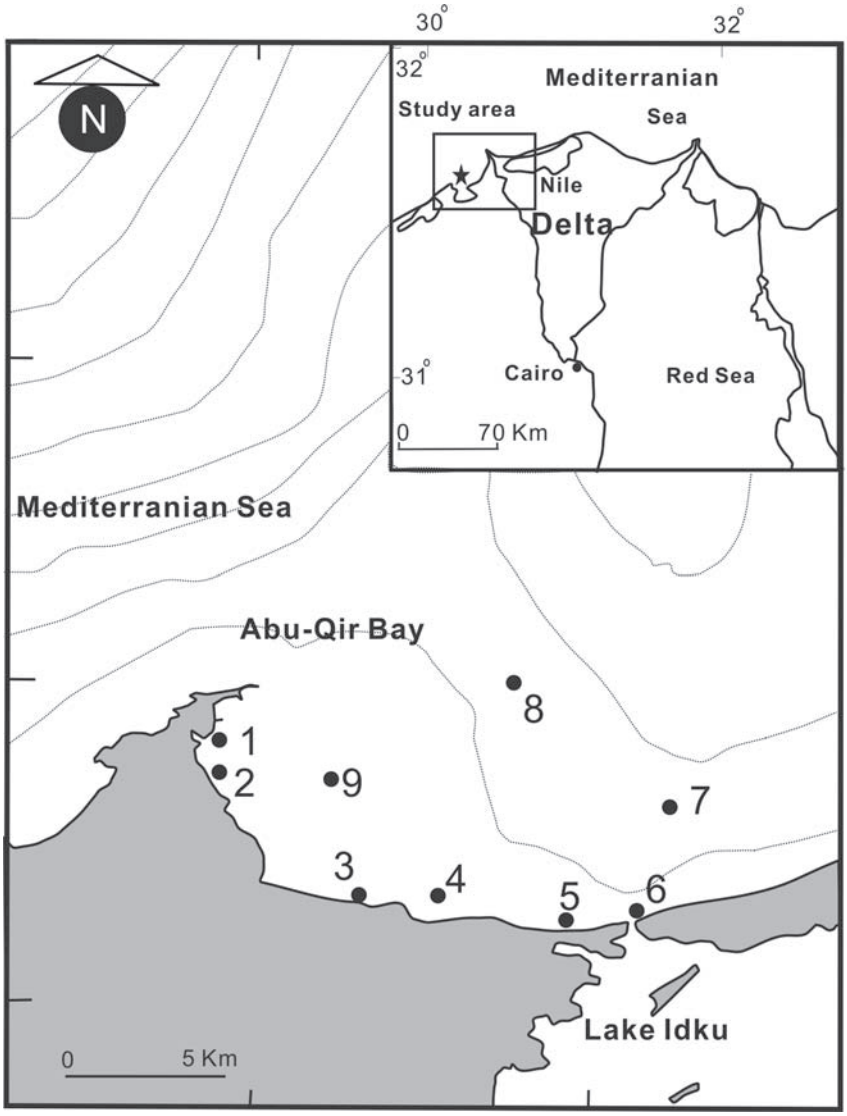

Figure 1. Study area and location of sampling stations.

In Abu-Qir Bay, the water temperature experiences the classical seasonal variations on the Egyptian Mediterranean coast (17°C–31.5°C). The surface salinity exhibits strong variations (6.42–39.05‰) depending on the variability in the volume of the discharged wastes. This discharge causes the occurrence of two distinguished water layers in the inner area of the bay; the low salinity surface layer and the subsurface sea water layer. The mixing of the two layers lead to the existence of three water masses in the bay with different salinities: <35‰, 35–38.55‰ and Mediterranean Sea water (>38.5‰). Dissolved oxygen displays low values (annual mean: 3.6 mg/L), but shows high variability (0.6–9.9 mg/L). The water transparency is low (0.3–4.0 m). High nutrient concentrations indicate a high eutrophication level, with a wide range of spatial and seasonal variations (Abdel Aziz et al., 2001).

Mean grain size generally decreases from west to east: a broad zone of medium to fine sand covers the western part of the bay; sediment becomes finer toward the east, and includes silt and clay-rich prodelta mud off the Rosetta promontory. Grain size also decreases from south to north, with fine sand along the coast, and mixtures of very fine sand, silt and clay in the bay and on the adjacent shelf (Frihy et al., 1994).

Table 1. Average concentrations of nutrients and metals in water discharged from Lake Edku into Abu-Qir Bay (Abdel-Moati, 2001). Abbreviations: TSM: total suspended matter, DIP: dissolved inorganic phosphate.

Variable	Lake Idku
TSM (mg/L)	87±43
DIP (μm/L)	4.12±3.1
NO_3^- (μm/L)	28.8±22
NH_4^+ (μm/L)	17.8±9.6
Pb (μg/L)	3.8±1.6
Cd (μg/L)	4.3±2.9
Hg (μg/L)	20±6

Current measurements in the bay indicated a NW current with a speed of 50 cm/sec in the inner bay and 5 cm/sec in the outer bay. At the lake-sea connection, there is a predominance of lake-bay flow throughout the year, with a maximum speed between 60 and 100 cm/sec along the axis of the channel. Tide in the bay is a typical semi-diurnal microtidal regime with a mean tidal range of 50 cm (UNESCO/UNDP, 1978).

3.3 Material and methods

3.3.1 Sampling

Eighteen surface sediment samples were collected from nine stations in Abu-Qir Bay by a grab sampler in two seasons during May and November 2005. The water depth of the sampled stations ranges from 0.83 to 8.88 m. The sites were selected according to different pollution levels as shown in Table (2). Locations of sample sites and regime are shown in Figure (1). At each site, 2 samples were collected from the surface sediments. The first one was stained and used for the study of foraminifera, while the second unstained one was subdivided into two parts from each site as follows: the first part was labelled and preserved for the grain size analysis and the second part was freezed to pass through geochemical analyses.

Sea surface temperature was measured at the bottom using a reverse thermometer attached to a Niskin water sampler. Salinity, pH and transparency were measured in situ by using portable salinometer, pH meter, and secchi disk respectively.

Table 2. Location and description of the selected stations.

Stations	Average depths (m)	Latitude	Longitude	Location and description
St.1	3.38	31° 19′ 068″	30° 04′ 285″	In front of Abu-Qir harbour.
St.2	0.83	31° 18′ 595″	30° 04′ 314″	To the south of the harbour, it is called Dead Sea.
St.3	1.15	31° 16′ 666″	30° 06′ 432″	Immediately down stream from El-Tabia pumping station, occasionally exposed to the effluent water.
St.4	1.81	31° 16′ 631″	30° 07′ 716″	In front of Abu-Qir Fertilizer Company.
St.5	3.38	31° 16′ 363″	30° 09′ 617″	In front of the Petrojet Company.
St.6	4.13	31° 16′ 35″	30° 10′ 200″	In front of the lake- sea channel (Boughaz El-Maadia), this station is mostly exposed to brackish water.
St.7	8.25	31° 18′ 45″	30° 11′ 15″	Offshore from St.6 and to the east of it.
St.8	8.88	31° 20′ 05″	30° 09′ 25″	Offshore from St.7 and St.9. It is the most offshore deeper station, it receives no discharge.
St.9	4.63	31° 18′ 029″	30° 06′ 140″	Offshore from El-Tabia station pumping

3.3.2 Methods

A constant volume (50 cm^3) was taken from the upper 0–3 cm of undisturbed surface sediments of each grab sample and stained with Rose Bengal ethanol solution for differentiating living from non-living foraminifera. In the laboratory, wet samples were sieved using 63 μm sieve to remove fine sediment particles, including silt and clay. The samples were dried in the oven at 60°C, and then kept in small bottles for subsequent analysis. Samples were then divided into splits, depending on the test concentration. Loeblich and Tappan's (1988) generic classification was followed and most species were determined by comparison with those of Cimerman and Langer (1991), Sgarrella and Moncharmont-Zei (1993), and Jones (1994). When possible, a minimum of 250-300 foraminiferal specimens were counted. All deformed tests, whenever present, were picked from each sample and morphologically examined. The standing stock of total benthic foraminifera is calculated and expressed as number of individuals per 50 cm^3. The relative abundance of each species was expressed as a percentage of the total assemblage. This data matrix was used for the statistical analyses.

Dissolved oxygen (DO) in water was determined according to Winkler method (Strickland and Parsons, 1975). The amount of dissolved oxygen is directly proportional to the titration of Iodine with a thiosulphate solution until a pale yellow colour of iodine is observed. Few drops of starch indicator are then added to give the blue colour of starch-iodine complex. The titration is continued until the disappearance of the blue colour.

To measure heavy metal concentrations in the sediment, about 60–80 mg of powdered dry sediment sample was digested in a microwave digestion vessel using nitric acid (9 ml), hydrochloric acid (2 ml), and hydrofluoric acid (3 ml) and the vessel was heated to 200°C. After cooling, the acid fumes were removed and the dry residue was dissolved in diluted nitric acid. The concentrations were measured using an inductively coupled plasma mass spectroscopy (ICP-MS). Eleven heavy metals were chosen for the analysis, lead (Pb), zinc (Zn), iron (Fe), copper (Cu), nickel (Ni), chromium (Cr), cadmium (Cd), manganese (Mn), vanadium (V), cobalt (Co), and scandium (Sc). The quality of the measurements were checked by comparing our

analysis of the certified reference material for marine sediment (MAG), which is a fine grained grey-brown clayey mud with low carbonate content, from the Wilkinson Basin of the Gulf of Maine, with its reference value (USGS, 1995).

A representative portion of the samples (about 20 gram) was used for grain size analysis using the standard dry sieving and sedimentation techniques (Krumbein and Pettijohn, 1938). Total organic carbon (TOC) was analysed using carbon analyser (C-200, version 2.6). Before analysis, the samples were dried and grounded. Calcium carbonate was calculated for all samples according to the following formula:

$$CaCO_3 = (TC\% - TOC\%) * 8.33$$

Where TC% is percentage of total carbon and TOC% is percentage of total organic carbon.

3.3.3 Statistical analyses

A Q-mode cluster analysis (sample by sample) was used to produce a dendrogram classification of samples from which sample associations are established. A similar cluster analysis was used to produce a dendrogram classification of dominant species (R-mode), from which species associations are formed. Only species with at least one occurrence of abundance in the analysed samples larger than 5% were included in the data matrix of data for the Q- and R-mode cluster analyses (Appendix 1). Samples were grouped according to their degree of similarity using the Euclidean distance for Q-mode and correlation coefficient for R-mode. Cluster analyses were performed using the Palaeontological Statistics Program (PAST), version 1.88.

Canonical Correspondances Analysis (CCA) was used to determine the community's relation to abiotic parameters at the sampled stations and was performed in CANOCO, version 4.02. We applied forward selection of variables and a Monte Carlo test based on 199 permutations to determine the significance of each environmental variable. On the CCA biplot, environmental variables are represented by arrows, which extend in both directions from the centre although only the positive direction is shown. The arrows point in the direction of maximum variation and length of the arrows demonstrates the relative importance of each environmental

variable. The centre of ordination diagram indicates the mean value for each environmental variable. The greater the angle between two environmental arrows, the less likely that they are related to one another. The perpendicular projection of species scores against these arrows allows for inferences to be made about the dominant environmental factors affecting species composition.

3.3.4 Diversity indices

To better evaluate the nature of the recognized assemblages, species diversity was estimated using the following three parameters:

1) *Dominance = 1 – Simpson index*. Ranges from 0 (all taxa are equally present) to 1 (one taxon dominates the community completely). $D = \Sigma (ni / n)^2$, where ni is number of individuals of taxon i.

2) *Simpson index = 1 – dominance*. Measures 'evenness' of the community from 0 to 1.

3) Shannon index: A diversity index, taking into account the number of individuals as well as number of taxa. Varies from 0 for communities with only a single taxon to high values for communities with many taxa, each with few individuals. $H = -\Sigma (ni / n) \ln (ni / n)$.

All of the above indices were calculated by means of the Palaeontological Statistics Program (PAST), version 1.88.

3.4 Results

3.4.1 Environmental parameters

The recorded data show marked seasonal variations of bottom water temperatures in the study area. Seasonally, the maximum and minimum bottom water temperatures are 25.5°C during spring and 21°C during autumn. Concerning the spatial variations, St.3 (El-Tabia pumping station) shows the highest bottom water temperature (25.5°C) in May, followed by St.4 (24.8°C) in May. Station 2 which is located in a semi-enclosed basin, is characterized by high temperature in both seasons (Table 3 and Fig. 2A).

Table 3. Spatial and seasonal variations of different environmental water parameters, organic matter, and carbonate content in the surface sediment in Abu-Qir Bay during the study period.

Station	Bottom temperature (℃)		Bottom salinity (‰)		Surface salinity (‰)		Bottom pH		Secchi depth (m)		Bottom DO (mg/L)		TOC (%)		$CaCO_3$ (%)	
	May	Nov.	May	Nov.	May	Nov.	May	Nov.	May	Nov.	May	Nov.	May	Nov.	May	Nov.
St.1	23.5	24.0	39.5	38.9	39.3	37.5	8.1	8.2	0.8	0.8	4.4	3.4	0.5	1.9	29.1	25.6
St.2	24.5	24.5	39.2	-	39.2	-	8.2	7.7	0.1	0.3	1.3	1.2	6.7	4.8	14.4	17.2
St.3	25.5	22.0	-	36.3	3.7	36.3	7.3	7.3	0.2	0.1	0.3	0.3	1.1	5.9	33.6	38.6
St.4	24.8	21.0	32.0	38.2	9.4	34.5	7.8	8.1	0.3	0.3	3.0	2.5	0.6	2.2	13.1	-
St.5	23.0	22.0	37.9	36.8	26.3	38.8	8.1	8.1	0.5	0.8	5.3	4.0	0.1	0.1	9.7	10.8
St.6	23.0	22.5	38.7	38.9	14.0	5.7	8.1	8.2	0.6	0.3	2.7	2.2	0.4	0.2	5.7	8.7
St.7	22.0	21.0	39.7	37.3	39.6	36.2	8.2	8.3	3.0	1.3	2.5	3.2	0.1	0.1	7.8	10.2
St.8	22.0	21.0	39.8	39.0	39.8	37.4	8.0	8.2	3.0	1.0	3.8	3.3	0.2	0.1	34.4	10.7
St.9	22.0	23.0	39.7	38.4	38.1	28.6	8.0	8.2	0.7	0.9	4.8	2.2	0.3	0.3	22.9	20.5

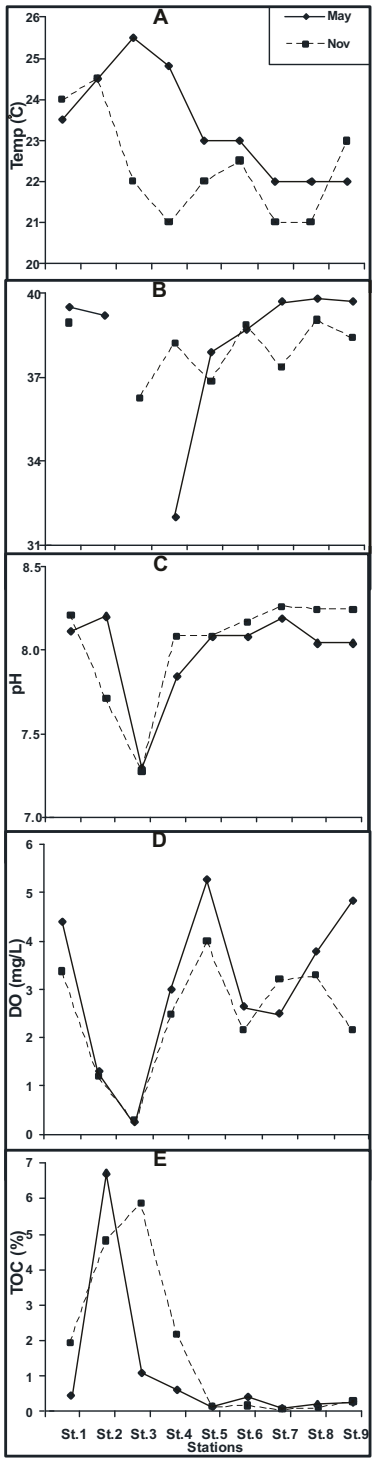

Figure 2. Regional and seasonal variations of bottom water temperature (°C) (A), salinity (‰) (B), pH (C), dissolved oxygen (mg/L) (D), and total organic carbon (%) of the sediment (E) in Abu-Qir Bay during the study period.

Variation of surface water salinity in Abu-Qir Bay is results from the interaction of sea water with brackish and polluted waters, discharged into this bay via El-Maadia

outlet and El-Tabia pumping station, respectively. The spatial salinity variations are more pronounced than the seasonal variations. Generally, the warmer season (May) is characterized by higher bottom water salinity than the colder season (November) in most of stations, with few exceptions (Table 3 and Fig. 2B). Station 6 is characterised by low surface salinity in both seasons (14.00‰ and 5.72‰). This is due to the effect of El-Maadia brackish discharge. Below this layer, the salinities increase with depth, the deeper bottom water (4.13 m) has salinity up to 38.89‰. Stations 3 and 4 have only low surface salinity in May, primarily reflecting the amount of the discharged water from El-Tabia pumping station and Abu-Qir Fertilizer Company. Again salinity increases downward at ~2 m to reach up to 38.21‰ at St.4 (Table 3).

During the study period, the pH of the bottom water was alkaline, ranging from 7.27 to 8.26. Spatially, St.3 (El-Tabia pumping station) has the lowest pH values in both seasons (7.27 and 7.3) (Table 3 and Fig. 2C). These low values are most probably due to the effect of the industrial discharge, which is enriched with organic matter. On the other hand, there is no marked seasonal variation of pH values. (Table 3 and Fig. 2C).

Transparency increased seaward and decreased near the entrance of the drains. The regional Secchi value in general is low at St.2, St.3, St.4 and St.6, with minimum values at St.3 in both seasons (0.1 m in November and 0.2 m in May) (Table 3). Seasonally, transparency has increasing trend in May and decreasing trend in autumn (Table 3). Low values are likely due to drain water discharged through El-Tabia pumping station, which is always characterized by its high turbidity. Low values are also related to suspended silt particles stirred up by water currents and enhanced winds in autumn, as well as to the nature of the discharged water, which was originally turbid and may be attributed to the shallowness of El-Tabia region. On the other hand, the highest value of transparency (3 m) is recorded at stable offshore stations (St.7 and St.8).

The concentration of DO in the bottom water of Abu-Qir Bay varies markedly from 0.26 mg/L to 5.28 mg/L (Table 3 and Fig. 2D). The lowest DO values in both seasons are measured at St.3 followed by St.2 (Table 3 and Fig. 2D). This could be attributed mainly to the consumption of DO by microbial decay of organic matter,

introduced to St.3 with the large quantities of industrial wastes and in St.2 by stagnant condition.

Sediments are mainly constituted of sand (up to 97.7%), less silt content (up to 19.6%), and insignificant clay content (up to 0.3%) (Table 4). The polluted discharge affects the constituents of the sediment in Abu-Qir Bay. Generally the polluted stations are characterised by the finest grain size. Accordingly, St.3 followed by St.2, St.6, and St.4 have the lowest percentages of sand (80.1%, 83.5%, 83.9%, and 86.2% respectively), and the highest percentages of silt (19.6%, 16.3%, 15.9%, and 13.6% respectively), while clay percentage is up to 0.3% (Table 4). From field observation St.3 was covered by a layer of sludgy material, caused by the disposal of the industrial wastes through El-Tabia pumping station. In contrast, the less polluted stations are characterized by high sand contents; the highest sand content is recorded in St.5, followed by St.8 and St.7 (Table 4).

Total organic carbon (TOC) concentrations show a wide range in the study area, with values ranging between 0.06% at St.7 in November and 6.7% at St.2 in May (Table 3 and Fig. 2E). There is a good correlation between TOC, DO and grain size. In finer sediment such as in St.2 and St.3, higher concentration of TOC (up to 6.7%) corresponds to low concentration of DO (minimum of 0.26 mg/L). The coarser sediments at St.5, St.7, St.8 and St.9 tend to have high DO (maximum of 5.28 mg/L) and less TOC concentration (minimum of 0.06%) (Table 3 and Fig. 2E). Moreover, TOC is decreasing markedly in the offshore direction in the less polluted stations (Table 3 and Fig. 2E). No pronounced seasonal TOC variation is recorded.

There are notable differences in the measured carbonate values in Abu-Qir Bay. The values fluctuate between 5.7% and 38.6%. Carbonate content tends to increase in the western part of the study area (e.g., St.1 and St.3), and decreases progressively toward the east (St.5 and St.6) (Table 3). This result is in agreement with Frihy et al. (1994). They indicated that two primary sediment sources have contributed to deposits in the study area. Calcareous-rich sediments in the most western part of the bay are derived from the carbonate ridge at the Abu-Qir headland and carbonate shelf province north and west of Alexandria. The second source of sediment is in front of El-Maadia channel derived from the reworking of clastic sediment from the former canopic distributary.

Geochemical results show high concentrations for all the measured heavy metals. The distribution patterns of the different heavy metal concentrations are more or less similar. The maximum concentrations of most of the measured heavy metals (e.g., Cd, Sc, V, Cr, Ni, Cu, and Zn) are recorded in St.2 (Dead Sea), followed by St.1 (harbour), St.3 (in the vicinity of El-Tabia pumping station), St.6 (in the vicinity of Boughaz El-Maadia), and St.4 (in the vicinity of Abu-Qir Fertilizer Company) with few exceptions (Table 4 and Fig. 3). Station 6 has a pronounced peak of Mn, Cr, V, and Co. Zinc tends to has high value at St.3 and St.4 whereas Mn, Fe, Co, and Pb show particular high value at St.1 (Table 4 and Fig. 3).

A high accuracy of our measurements is documented by an excellent fit of our own analyses of reference material for marine sediment (MAG) with certified values (USGS, 1995) (Table 4). The results have been compared with published reference values of Mediterranean coastal areas (Damiani et al., 1987; El-Sayed et al., 1988; Rifaat et al., 1992; El-Sayed and Rifaat, 1993), background values of the natural unpolluted beach located west of Alexandria (Rifaat and Deghedy, 1996) have been carried out. Rifaat and Deghedy (1996) found that sediments of their study area contained lower average concentrations of metals when compared to other areas in the Mediterranean Sea (El-Sayed et al., 1988; Rifaat et al., 1992; El-Sayed and Rifaat, 1993). In the present study, almost all the parameters, except Cd that show very low concentrations (Table 4), exceed the reference values. Particularly, Zn (up to 434.8 ppm), Cu (up to 103.4 ppm), and Cr (up to 133.7 ppm) exceed the natural background levels more than ten times in many stations located near the industrial plant. Surprisingly, St.8, the most faraway station from the pollution source, exceeds the natural background levels for some heavy metals (e.g., Zn and Cr). Obviously, the pollution in Abu-Qir Bay extends even to the offshore stations. A comparison with previous heavy metal studies in Abu-Qir Bay (Saad et al., 1980) indicates that all heavy metals concentrations increased markedly since 1980 until present (Table 4).

Table 4. Spatial variations of heavy metal (ppm), and grain size (%) of the surface sediment in Abu-Qir Bay.

Stations	sand%	silt%	clay%	Cd	Pb	Sc	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
St.1	87.7	12.1	0.2	0.33	49.13	12.92	109.72	120.70	674.12	45314.64	15.89	44.05	74.86	155.94
St.2	83.5	16.3	0.2	0.55	44.26	14.24	114.14	130.44	541.85	40974.62	15.09	47.11	111.41	300.11
St.3	80.1	19.6	0.3	0.54	31.51	9.51	87.16	102.97	446.16	27265.19	12.63	37.61	103.40	434.81
St.4	86.2	13.6	0.2	0.25	14.98	6.29	56.94	76.61	383.28	18624.79	8.49	24.19	37.21	383.18
St.5	97.7	2.1	0.2	0.05	5.53	3.11	23.52	32.36	300.85	8169.50	5.03	7.52	3.70	14.14
St.6	83.9	15.9	0.2	0.18	6.16	9.50	104.08	133.78	636.46	26375.63	12.52	24.05	11.09	53.46
St.7	93.5	6.4	0.1	0.05	3.42	2.65	19.44	26.17	387.43	6093.72	3.20	5.13	2.12	9.02
St.8	94.0	5.8	0.2	0.05	4.92	3.41	25.05	29.64	305.77	8814.95	5.36	8.09	3.67	13.65
St.9	91.3	8.4	0.3	0.10	7.23	4.87	36.25	67.38	338.66	9794.04	4.45	9.31	4.93	21.93
Mean (present study)				0.23	18.57	7.39	64.03	80.01	446.06	21269.68	9.19	23.01	39.15	154.03
Mean (1980) a				2.02					45	4500			12	102
Background b				0.90	5.20			5.70					3.90	3.40
Damiani and others (1987) c					60			30	700	25,000		20	20	80
USGS (1995) d				0.20 ±0.03	24 ±3	17 ±1	140 ±6	97 ±8			20 ±1.6	53 ±8	30 ±3	130 ±13
MAG e				0.27	26.55	17. 93	147.50	102.28			21.40	50.78	26.93	124.78

a: Mean heavy metal concentrations in previous study in Abu-Qir Bay (Saad and others, 1980).
b: Background concentrations for the study area (Rifaat and Deghedy, 1996).
c: Data from other Mediterranean region (Damiani and others, 1987).
d: Certificate of Analysis by USGS (1995).
e: Our laboratory analysis of certified reference material (MAG).

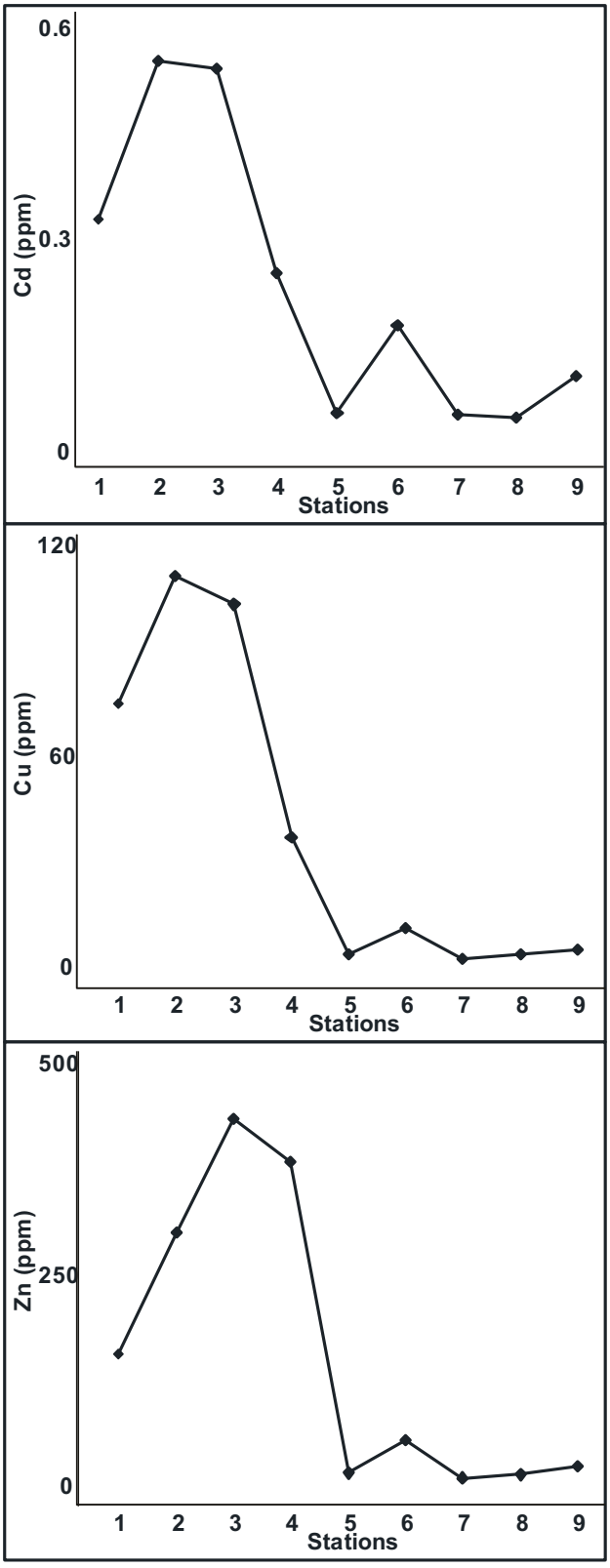

Figure 3. Heavy metal concentrations (ppm) of the surface sediment in Abu-Qir Bay.

3.4.2 Benthic Foraminifera

3.4.2.1 Benthic foraminiferal density and diversity

The standing crop of benthic foraminifera in Abu-Qir Bay is generally high and ranges from 66015 individuals per 50 cm^3 at St.2 Nov. to 7065 individuals per 50 cm^3 at St.5 Nov. (Table 5). The density of foraminifera generally increases in stations polluted by organic matter, especially in St.2, St.3, and St.6. There are no clear seasonal foraminiferal density contrasts (Table 5 and Fig. 4).

Station 2 is characterised by the lowest diversity in both seasons as expressed by low values of the Shannon index (1.54, 1.52), and Simpson index (0.66, 0.66) in May and November respectively (Table 5 and Fig. 4). On the other hand, percentage dominance values show the reverse pattern to species diversity, hence St.2 has the highest dominance value (Table 5 and Fig. 4). This high value indicates that only a few species were present in high abundance (mostly *Ammonia tepida*). Other polluted stations such as St.1, St.3, St.4, and St.6 follow more or less the same low diversity trend of St.2. Conversely, the fauna of St.8 followed by St.9, St.7, and St.5 exhibit the highest diversities and the lowest dominance values. Species richness is higher within less polluted areas, where the number of species could be nearly threefold that of the more polluted areas. For example, 26 species are recorded in St.2, one of the most polluted areas, while 59 species are counted in St.8 Nov. (Table 5). In general, diversities increase with increasing water depth and decreasing pollution. In the study area the foraminiferal density shows more or less a reverse pattern with density. Station 2 and St.3 show the highest foraminiferal densities and the lowest diversity. The vice versa is recorded in offshore stations (Table 5 and Fig. 4). The average seasonal values of all faunal parameters were displayed in the figures, since there are no clear systematic temporal contrasts.

Table 5. Foraminiferal data and diversity indices at sampled stations during two sampling periods in Abu-Qir Bay.

Stations	Rotaliina (%)	Miliolina (%)	Deformed forms (%)	Living individuals %	Species number	Dominance	Simpson	Shannon	Density
St.1 May	74.02	25.78	2.45	8.46	49	0.09	0.91	2.89	18534
St.1 Nov.	55.13	44.02	0.43	32.39	34	0.18	0.82	2.32	28600
St.2 May	56.60	43.40	1.67	66.01	26	0.34	0.66	1.54	43109
St.2 Nov.	54.43	45.57	2.01	49.70	27	0.34	0.66	1.52	66016
St.3 May	76.00	24.00	0.94	48.49	42	0.20	0.80	2.52	30781
St.3 Nov.	65.35	34.65	1.52	45.85	32	0.13	0.87	2.55	55677
St.4 May	67.88	31.20	0.83	50.92	39	0.11	0.89	2.87	18181
St.4 Nov.	79.00	20.57	0.00	51.95	32	0.14	0.86	2.62	27821
St.5 May	63.18	36.82	0.33	38.79	36	0.09	0.91	2.81	13194
St.5 Nov.	58.80	40.70	0.00	41.71	46	0.05	0.95	3.31	7065
St.6 May	53.75	45.83	0.70	35.42	47	0.07	0.93	3.19	46787
St.6 Nov.	58.70	40.00	0.74	66.52	47	0.08	0.92	3.16	36734
St.7 May	36.93	61.93	0.00	31.25	52	0.04	0.96	3.53	20058
St.7 Nov.	55.96	44.04	0.00	46.15	35	0.08	0.92	2.91	8620
St.8 May	45.70	54.30	0.00	58.18	49	0.04	0.96	3.48	13787
St.8 Nov.	48.65	50.90	0.00	75.68	59	0.03	0.97	3.74	15640
St.9 May	47.17	52.52	0.00	72.33	57	0.05	0.95	3.43	27569
St.9 Nov.	40.91	58.71	0.46	55.30	56	0.05	0.95	3.53	23384

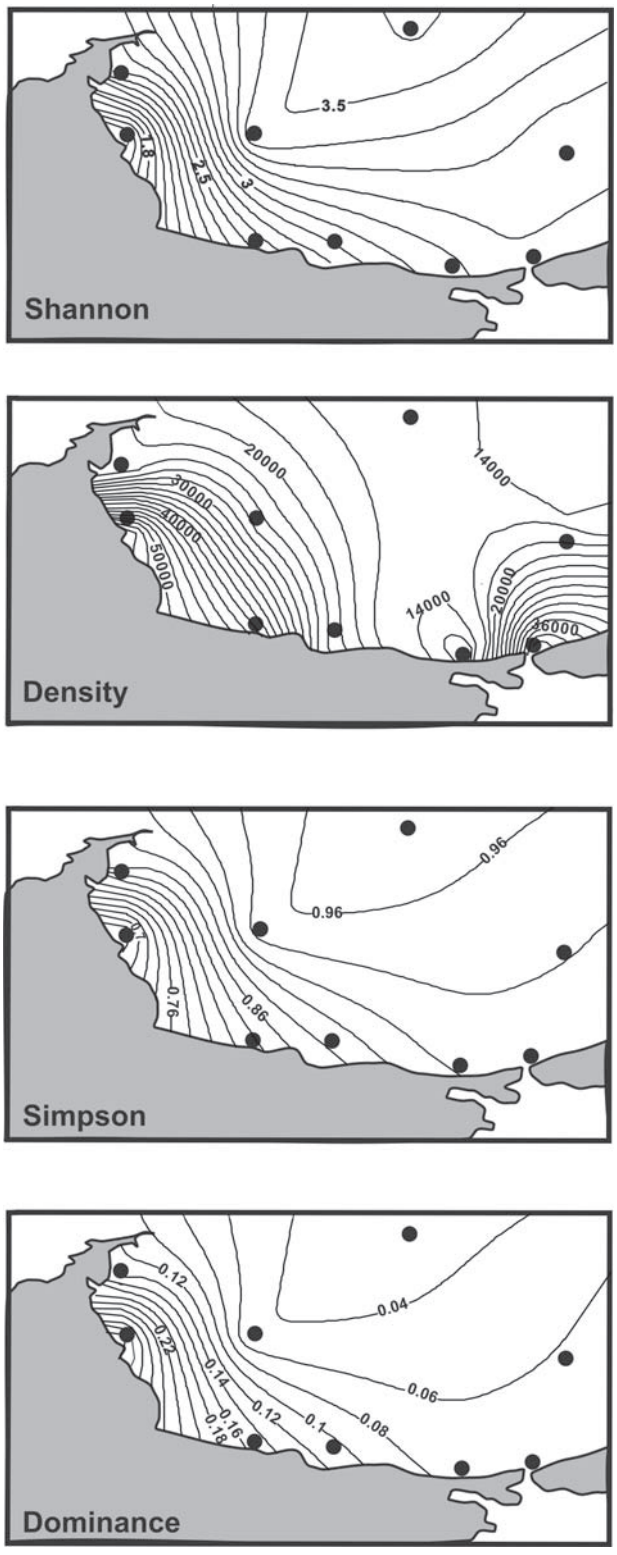

Figure 4. Pattern of different diversity indices and total foraminiferal abundances in the studied samples. Since there are no clear systematic temporal variations, the average seasonal values of faunal diversity indices are displayed in the figures.

3.4.2.2 Distribution of benthic foraminiferal indicator species and faunas

A total of 105 benthic foraminiferal species belonging to 47 genera are identified throughout the study area (Appendix 2, Figs. 5-9). Seventeen species with a relative abundance exceeding 5% of the assemblage in at least one sample were used for statistical analyses. Among them 6 taxa are the most abundant. Their relative abundances over all stations and sampling periods in decreasing order are: *Ammonia tepida* (19.5%), *Quinqueloculina lata* (12.3%), *Quinqueloculina* spp. (9.2%), *Asterigerinata mamilla* (4.9%), *Elphidium* spp. (3.6%), *and Ammonia beccarii* (2.7%).

The suborder Rotaliina constitutes the highest percentages among the total foraminifera, representing 58.6% of the total assemblages in the study area. It is mainly represented by members of the family Rotaliidae. The most abundant species is *A. tepida*, constituting 19.5% of the total foraminiferal association. This could be attributed to their ability to colonize in polluted areas as will be discussed later. Other major species in this suborder present are *A. mamilla* (5.0%), *Elphidium* spp. (3.6%), *A. beccarii* (2.7%), *Cibicides refulgens* (2.7%), and *Brizalina* spp. (2.4%). The suborder Miliolina is the second most important component in the recorded foraminiferal assemblages, comprising 41.1% of the total assemblages in Abu-Qir Bay. The second most abundant species among all foraminifera recorded is *Q. lata*, constituting 12.3% of the total foraminiferal fauna, followed by *Quinqueloculina* spp. (9.2%), *Spiroloculina* spp. (1.5%), *Quinqueloculina stelligera* (1.4%), *Quinqueloculina vulgaris* (1.4%), *Quinqueloculina viennensis* (1.3%), and *Triloculina trigonula* (1.3%). Rotaliina dominate the shallower and polluted stations such as St.3, St.4 in both seasons and St.1 in May (up to 79%), while Miliolina dominate the deeper water stations (St.7, St.8, and St.9 in both seasons, especially St.7 May (61.9%) and St.9 in Nov. (58.7%) (Table 5 and Fig. 10). Minor components of the recorded assemblages were represented by members belonging to the suborders Lagenina (0.2%), and Spirillinina (0.1%). Agglutinated species are very rare (Textulariina, 0.02%) in Abu–Qir Bay.

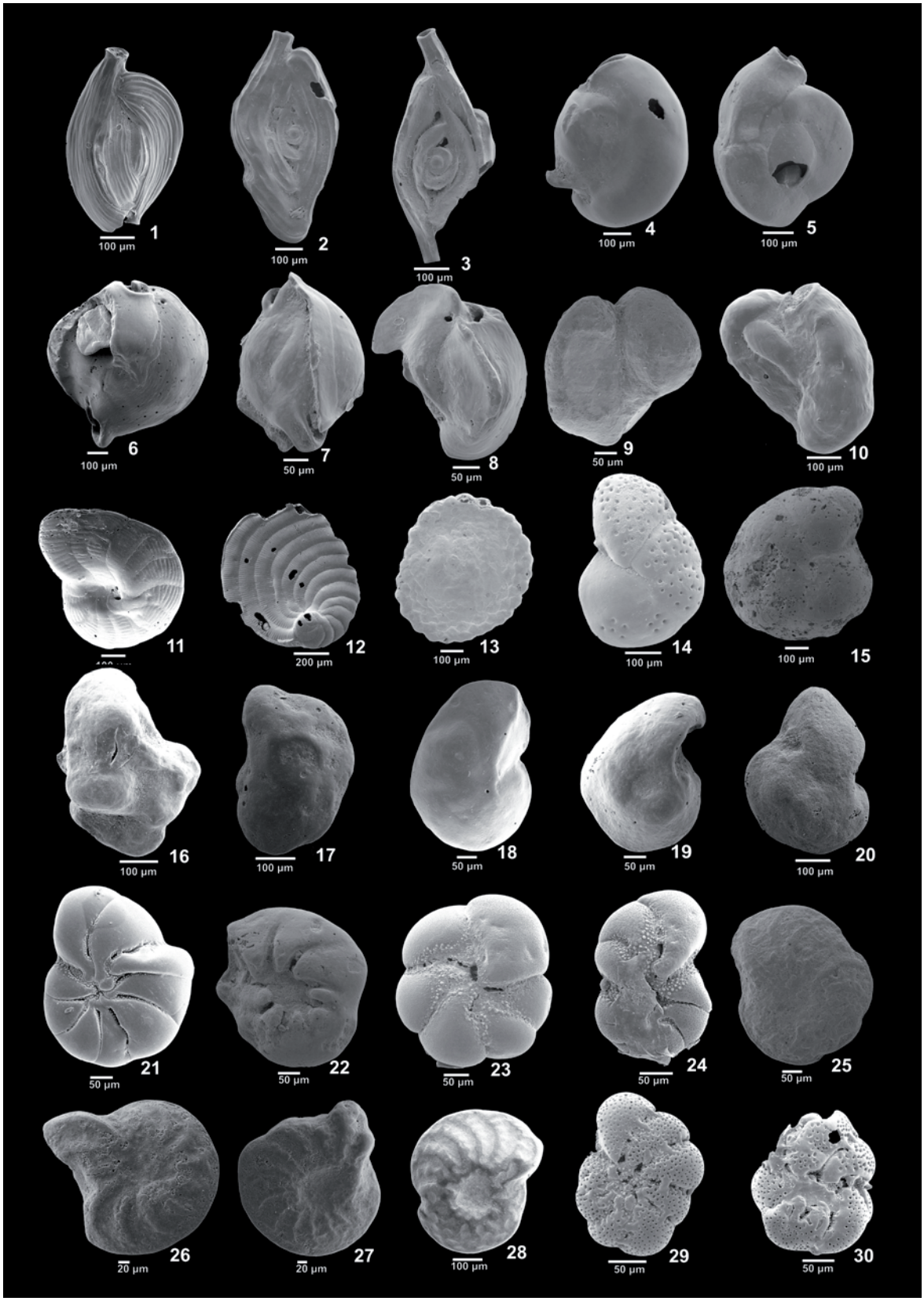

Figure 5 Deformed foraminiferal specimens from Abu-Qir bay, Egypt.

5.1. *Adelosina mediterranensis* (Le Calvez J. and Y., 1958). Abnormal specimen shows two apertures. 5.2. *Spiroloculina* spp., side view of deformed specimen indicates abnormal growth (wavy chamber). 5.3. *Spiroloculina* spp., side view of biaperturate deformed specimen. 5.4. *Lachlanella* spp. side view of deformed specimen shows hole and abnormal growth. 5.5. *Quinqueloculina* spp., side view of deformed specimen shows division of the last chamber and discontinuity of growth. 5.6. *Quinqueloculina* spp., side view of deformed specimen indicates 2 apertures and microboring. 5.7. *Quinqueloculina* spp., deformed side view shows 2 apertures. 5.8. *Quinqueloculina* spp., side view of deformed specimen shows abnormal growth and flaring last chamber. 5.9. *Quinqueloculina* cf. *Q. vulgaris* (d'Orbigny, 1826), side view of deformed specimen shows separation of chambers (loose coiling). 5.10. *Miliolinella* spp., side view of deformed specimen shows abnormal growth. 5.11. *Peneroplis pertusus* (Forskäl, 1775). Side view shows abnormal unwadged reduced chamber. 5.12. Deformed forms of *Peneroplis planatus* (Fichtel and Moll, 1798). Note the abnormal growth of the test. 5.13. *Sorites orbiculus* (Ehrenberg, 1839), side view of deformed specimen shows abnormal growth. 5.14. *Cibicides lobatulus* (Walker and Jacob, 1798), spiral side of deformed specimen indicates elongated periphery due to elongated (flaring) last chamber. The deformation in *Cibicides* species could be due to substrate effect rather than pollution effect. 5.15. *Cibicides refulgens* (Montfort, 1808), spiral view of deformed specimen shows small ultimate (or final) chamber. 5.16. Highly deformed specimens of *Cibicides refulgens* (Montfort, 1808). 5.17. *Cibicides refulgens* (Montfort, 1808), spiral side of deformed specimen shows elongated periphery rather than rounded, ultimate chamber protruding. 5.18. Deformed forms of *Cibicides refulgens* (Montfort, 1808), note that the periphery becomes not rounded because of the protruding ultimate and penultimate chambers. 5.19-5.20. Spiral view of *Cibicides refulgens* (Montfort, 1808) shows flaring last chamber. 5.21. *Astrononion stelligerum* (d'Orbigny, 1839). Side view of deformed specimen shows disturbance in growth before pen ultimate and pen chambers. 5.22. *Ammonia* spp., umbilical view of deformed specimen shows abnormal periphery of the test. 5.23-5.24. Deformed forms of *Ammonia tepida* (Cushman, 1926). (5.23) Side view shows deformation in umbilical side (not typical wedge shape of chamber). (5.24) Side view indicates abnormal growth. 5.25. Deformed forms of *Elphidium* cf. *E. advenum* (Cushman, 1922); note the elongated ultimate chamber, consequently not rounded periphery and dissolution. 5.26. *Elphidium* spp. side view of deformed specimen shows protruding last chamber. 5.27-5.28. *Elphidium* spp. Side view of deformed specimen indicates abnormal growth. 5.29-5.30. *Porosononion* spp. (5.29) Side view of deformed specimen shows deformation in size, irregular periphery (not rounded), some chambers are not in a suitable size. (5.30) Deformed specimen shows abnormal growth and microboring.

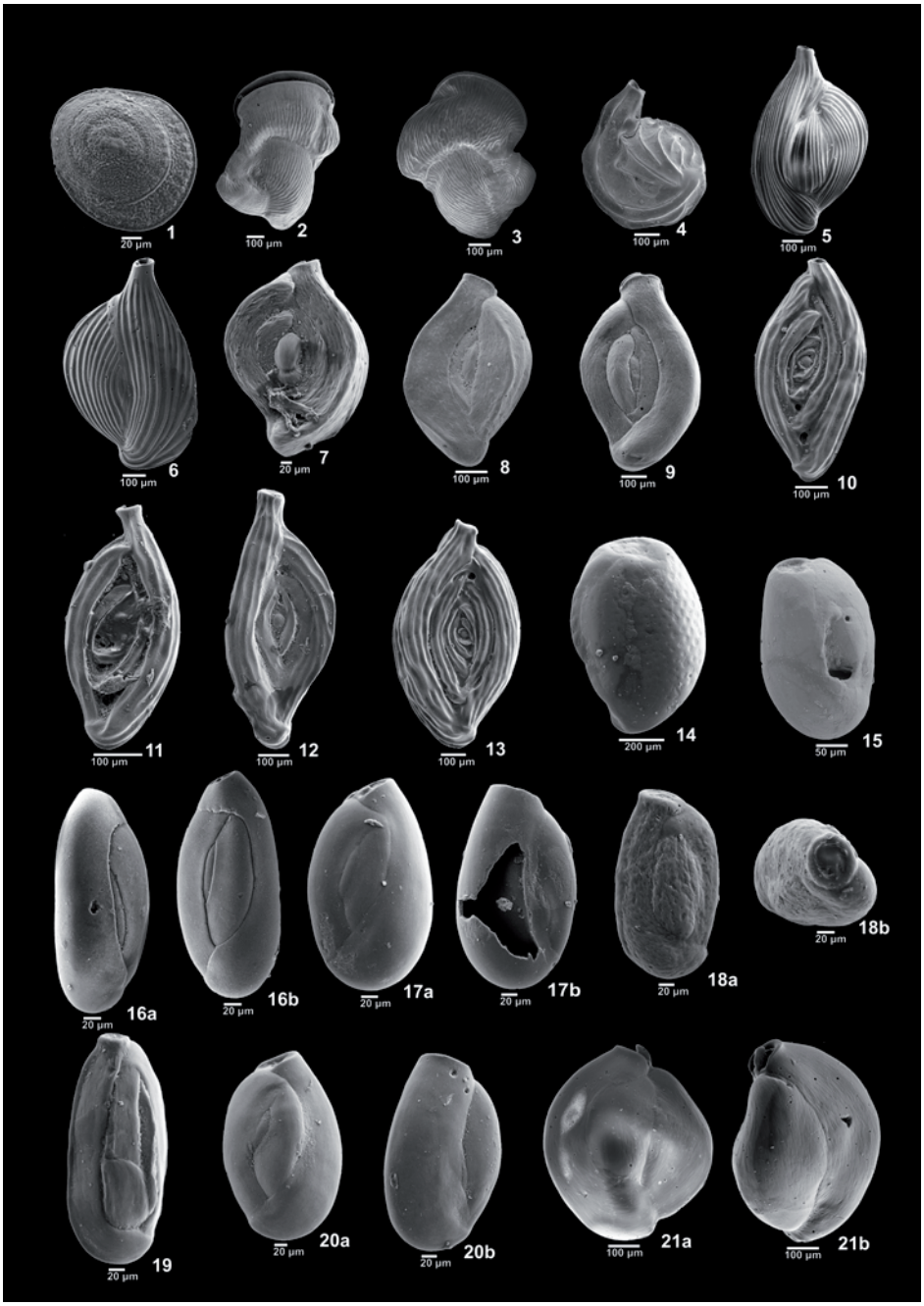

Figure 6. SEM images of a selection of the foraminiferal species from Abu-Qir bay, Egypt. 6.1. *Patellina corrugata* (Williamson, 1858). Spiral view. 6.2-6.3. *Vertebralina striata* (d'Orbigny, 1826). Side view. 6.4. Juvenile stage of *Adelosina mediterranensis* (Le Calvez J. and Y., 1958). Side view. 6.5-6.6. *Adelosina mediterranensis* (Le Calvez J. and Y., 1958). Side view. 6.7. *Spiroloculina* cf. *S. dilatata* (d'Orbigny, 1846). Side view. 6.8. *Spiroloculina nummiformis* (Said, 1948). Side view. 6.9. *Spiroloculina rotundata* (d´Orbigny, 1826). Side view. 6.10-6.13. *Spiroloculina* spp. Side view. 6.14. *Lachlanella variolata* (d'Orbigny, 1826). Side view. 6.15. *Quinqueloculina* spp. (d'Orbigny, 1839) side view of normal specimen shows big hole. 6.16. *Quinqueloculina laevigata* (d'Orbigny, 1839). (6.16a) 3 chamber side, (6.16b) 4 chamber side. 6.17. *Quinqueloculina lata* (Terquem, 1876). (6.17a) 4 chamber side, (6.17b) 3 chamber side. 6.18. *Quinqueloculina parvula* (Schlumberger, 1894). (6.18a) 4 chamber side, (6.18b) Apertural view. 6.19. *Quinqueloculina stelligera* (Schlumberger, 1893). 4 chamber side. 6.20. *Quinqueloculina* cf. *Q. viennensis* (le Calvez and le Calvez, 1958). (6.20a) 4 chamber side, (6.20b) 3 chamber side. 6.21. *Quinqueloculina vulgaris* (d'Orbigny, 1826). (6.21a) 4 chamber side, (6.21b) Apertural view.

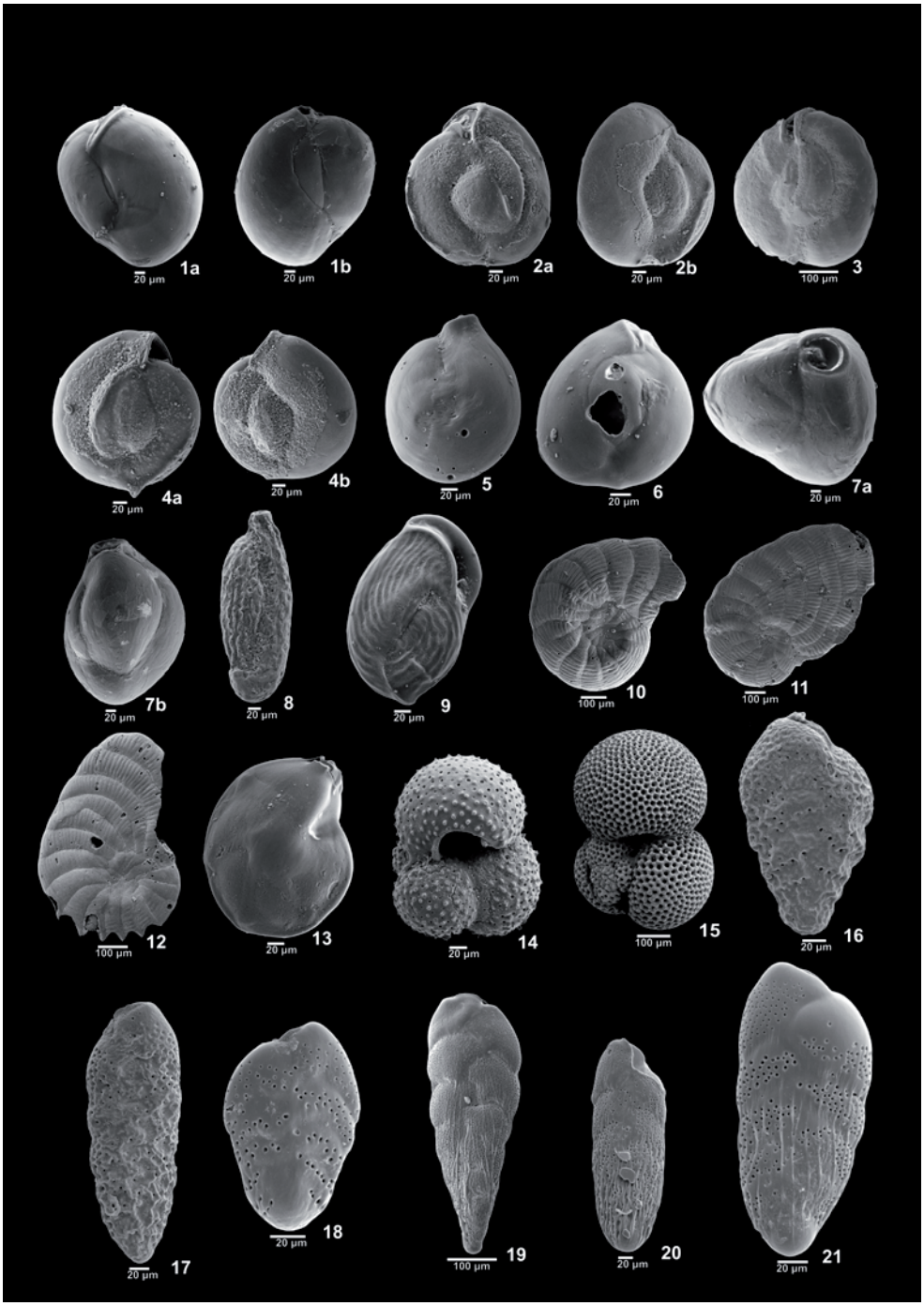

Figure 7. SEM images of a selection of the foraminiferal species from Abu-Qir bay, Egypt. 7.1a-7.1b. *Miliolinella labiosa* (d'Orbigny, 1839). Side view. 7.2a-7.2b. *Miliolinella semicostata* (Wiesner, 1923). Side view. 7.3. *Miliolinella semicostata* (Wiesner, 1923). Side view. 7.4a-7.4b. *Miliolinella subrotunda* (Montagu, 1803). Side view. 7.5. *Miliolinella subrotunda* (Montagu, 1803). Side view. 7.6. *Miliolinella* spp. Side view. 7.7. *Triloculina trigonula* (Lamarck, 1804). (7.7a) Apertural view, (7.7b) Front view. 7.8. *Sigmoilinita costata* (Schlumberger, 1893). Side view. 7.9. *Articulina mucronata* (d'Orbigny, 1839). Side view. 7.10. *Peneroplis pertusus* (Forskål, 1775). Side view. 7.11. *Peneroplis planatus* (Fichtel and Moll, 1798). Side view. 7.12. *Peneroplis planatus* (Fichtel and Moll, 1798), side view of normal specimen shows dissolution and microperforation. 7.13. *Lenticulina* spp. Side view. 7.14. *Globigerina bulloides* (D'orbigny, 1826) (planktonic species). Side view. 7.15. *Globigerinoides* sacculifer **(**Brady, 1877) (planktonic species). Side view. 7.16-7.17. *Bolivina variabilis* (Williamson, 1858). Side view. 7.18. *Brizalina dilatata* (Reuss, 1850). Side view. 7.19-7.21. *Brizalina striatula* (Cushman, 1922). Side view.

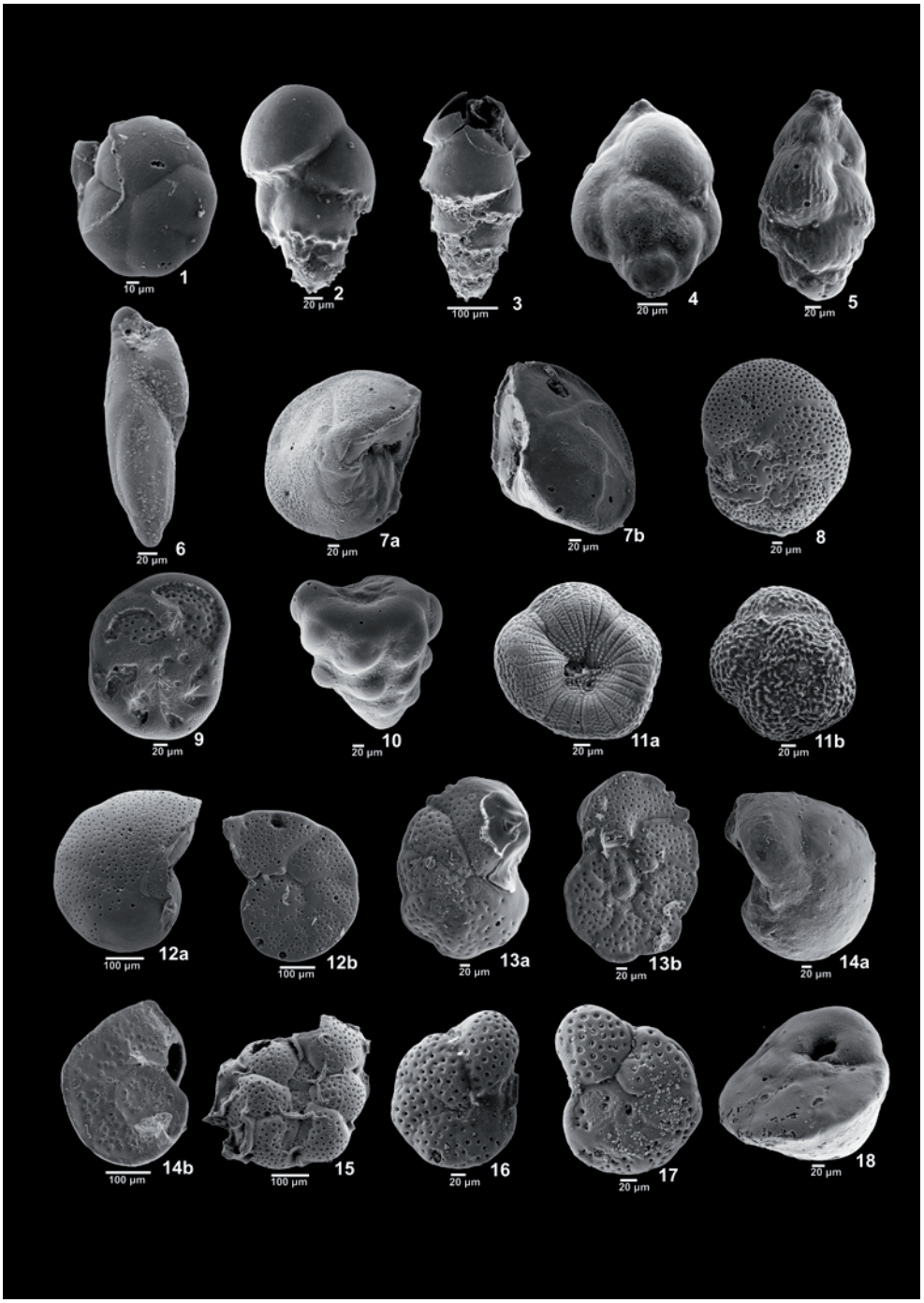

Figure 8. SEM images of a selection of the foraminiferal species from Abu-Qir bay, Egypt. 8.1. *Cassidulina crassa* (d'Orbigny, 1839). Side view. 8.2. *Bulimina aculeata* (d'Orbigny, 1826). Side view. 8.3. *Bulimina marginata* (d'Orbigny, 1826). Side view. 8.4. *Bulimina* spp. Side view. 8.5. *Siphouvigerina* spp. Side view. 8.6. *Fursenkoina* spp. Side view. 8.7. *Eponides concamerata* (Williamson, 1858). (Figure 8.7a) Spiral side, (Figure 8.7b) Apertural edge view. *Rosalina bradyi* (Cushman, 1915). Spiral side. 8.9. *Rosalina macropora* (Hofker, 1951). Spiral side. 8.10. *Glabratella erecta* (Sidebottom, 1908). Side view. 8.11. *Glabratella hexacamerata* (Seigle and Bermudez, 1965). (8.11a) Umbilical side, (8.11b) Spiral side. 8.12. *Cibicides lobatulus* (Walker and Jacob, 1798). (8.12a) Spiral side, (8.12b) Uumbilical side. 8.13. *Cibicides lobatulus* (Walker and Jacob, 1798). (8.13a) Spiral side, (8.13b) Uumbilical side. 8.14. *Cibicides refulgens* (Montfort, 1808). (8.14a) Spiral side, (8.14b) Umbilical side. 8.15. *Planorbulina mediterranensis* (d'Orbigny, 1826). Unattached side view. 8.16. *Cibicidella variablis* (d'Orbigny, 1839). Spiral side. 8.17. *Cibicidella variablis* (d'Orbigny, 1839). Umbilical side. 8.18. *Asterigerinata mamilla* (Williamson, 1858). Umbilical side.

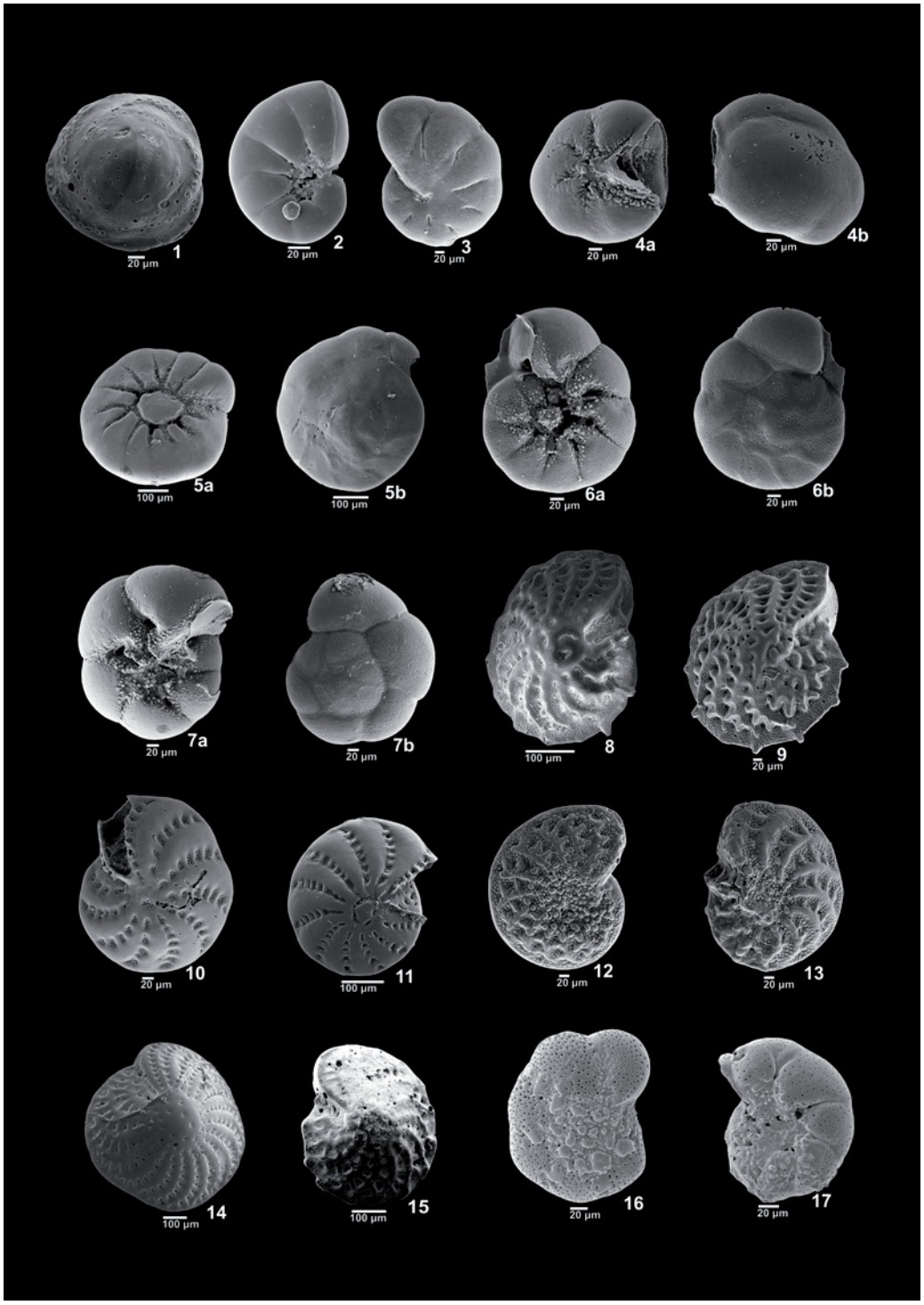

Figure 9. SEM images of a selection of the foraminiferal species from Abu-Qir bay, Egypt. 9.1. *Asterigerinata mamilla* (Williamson, 1858). Spiral side. 9.2. *Nonion* cf. *N. commune* (Kassel, 1963). Side view. 9.3. *Astrononion stelligerum* (d'Orbigny, 1839). Side view. 9.4. *Buccella* spp. (9.4a) Umbilical side, (9.4b) Spiral side. 9.5. *Ammonia beccarii* (Linnaeus, 1758). (9.5a) Umbilical side, (9.5b) Spiral side. 9.6. *Ammonia* cf. *A. parkinsoniana* (d'Orbigny, 1839) (9.6a) Umbilical side, (9.6b) Spiral side. 9.7. *Ammonia tepida* (Cushman, 1926). (9.7a) Umbilical side, (9.7b) Spiral side. 9.8-9.9. *Elphidium aculeatum* (d'Orbigny, 1846). Side view. 9.10-9.11. *Elphidium* cf. *E. advenum* (Cushman, 1922). Side view. 9.12-9.13. *Elphidium complanatum* (d'Orbigny, 1839). Side view. 9.14. *Elphidium crispum* (Linnaeus, 1758). Side view. 9.15. *Elphidium* cf. *E. crispum* (Linnaeus, 1758). Side view. 9.16-9.17. *Porosononion* spp. Side view.

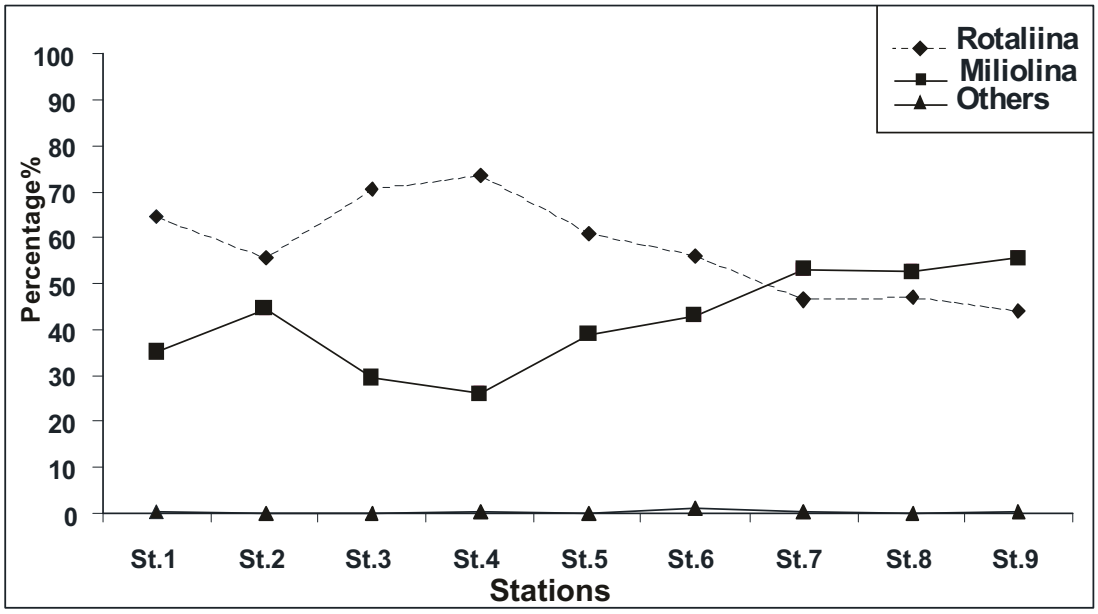

Figure 10. Distribution of the major foraminiferal suborders in the study area. For simplicity the average seasonal values of faunal distribution are displayed in the figure.

Due to the shallow water environments in the bay, planktic foraminiferal species were very rare. Their occurrence is considered as transported with currents and waves from offshore into coastal waters. They are represented by members of the suborder Globigerina (family Globigerinidae), e.g., *Globigerinoides sacculifer, Globigerina bulloides*, and *Globigerinoides* spp. The number of planktonic foraminiferal tests increases in November, probably related to increased wind action and coastal currents.

The highest percentage of living foraminifera is recorded in St.8 Nov. (75.7%) and St.9 May (72.3%) (Table 5 and Fig. 11). The harbour station (St.1) is characterized by the lowest percentage of living foraminifera (8.5%) in May. In addition to the high pollution level at this station, the foraminiferal assemblages have been affected by the agitation of currents and waves generated by ship action, resulting in the reworking of foraminiferal tests. These processes create an unstable environment that is not favourable for the production of a rich live fauna. Moderate to high relative abundances of living specimens are recorded in these stations, especially in St.2 (66.0% and 49.7% in both seasons) (Table 5 and Fig. 11). Although it is expected that due to heavy metal pollution, the proportion of living specimens are very few in

other polluted stations, This is related to the tolerance and dominance of living *A. tepida* in this station.

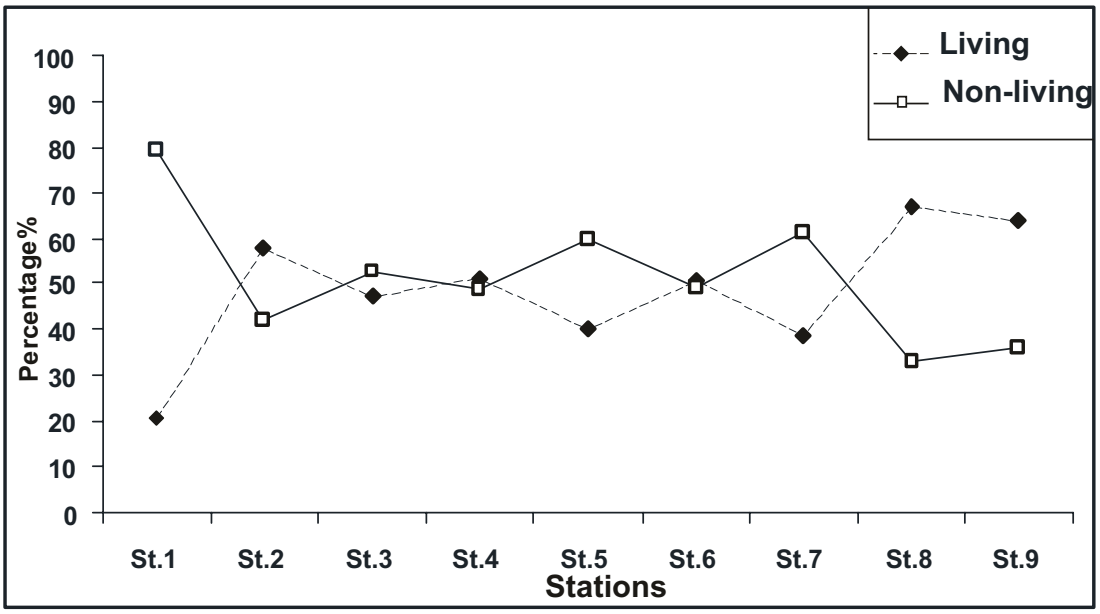

Figure 11. Distribution pattern of living and non-living foraminifera in the studied samples. For simplicity the average seasonal values of faunal distribution are displayed in the figure.

The temporal and spatial distribution of the 6 most abundant species will be addressed in detail. *Ammonia tepida* is the most abundant taxon in the study area. Its distribution is coincident with the pollution pattern. Its relative abundance increases in onshore polluted stations with organic-rich fine grained substrates (St.2, St.3, St.4, St.6 and St.1) (Fig. 12A). It has a very pronounced peak (up to 42.4%) in the low salinity**,** semi-closed highly polluted basin (St.2). It decreases sharply in saline non-polluted /offshore stations with coarse sediment (St.5, St.7, St.8, and St.9). *Quinqueloculina lata*, the second most dominant taxon, has more or less similar distribution to *A. tepida* with a very pronounced peak is St.2, followed by other polluted stations (Fig. 12B). The relative abundance of *Quinqueloculina* spp. fluctuates all over the stations, with low values in St.2, St.3 and St.4 and high values in St.7 and St.5 (Fig. 12C). The distribution of *A. mamilla* anticorrelates with that of *A. tepida*. High relative abundances occur in offshore coarse sediments at stations St.5, St.7, St.8, St.9, and especially St.5 (Fig. 12D). High numbers of empty tests were also found at St.1, likely due to current transport at this site. *Elphidium* spp. has

a very similar distribution pattern when compared to that of *A. mamilla*, with low numbers at the near-shore polluted stations and high numbers at offshore/non-polluted stations, especially at St.7 and St.5 (Fig. 12E). Similar to *A. mamilla*, high numbers of dead *Elphidium* spp. are also found at St.1. The relative abundance of *A. beccarii* undergoes variations among stations. The highest values are recorded at St.9, and St.7 (Fig. 12F). The spatial variability of the major foraminiferal taxa clearly exceeds the seasonal contrasts.

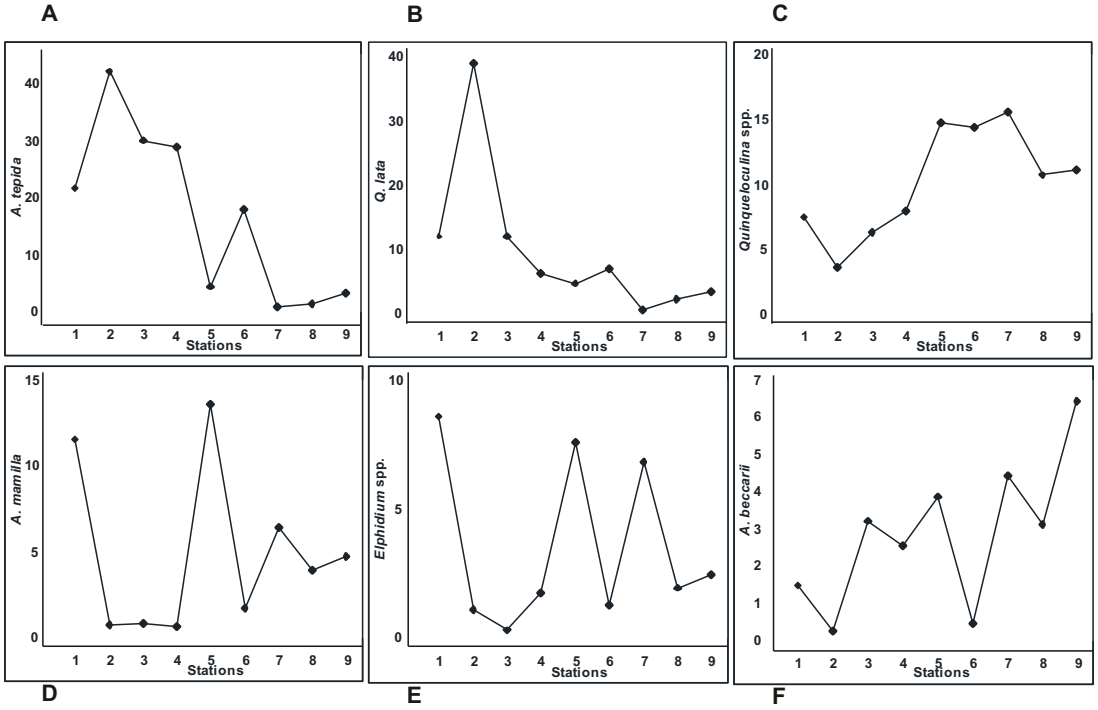

Figure 12. Distribution pattern of the 6 most dominant foraminiferal species expressed as relative abundance. *Ammonia tepida* (A), *Quinqueloculina lata* (B), *Quinqueloculina* spp. (C), *Asterigerinata mamilla* (D), *Elphidium* spp. (E), and *Ammonia beccarii* (F). For simplicity, the average seasonal values of faunal parameters are displayed in the figure.

3.4.3 Relation between benthic foraminifera and environmental condition as indicated from statistical analyses

3.4.3.1 Results of the Cluster analysis (CA)

In the study area, the CA has revealed distinct clusters, characterised by different environmental types and degrees of pollution. Q-mode cluster analysis was

performed to study similarities between the stations. Samples are grouped into two main clusters (A and B), each with two subclusters (Fig. 13).

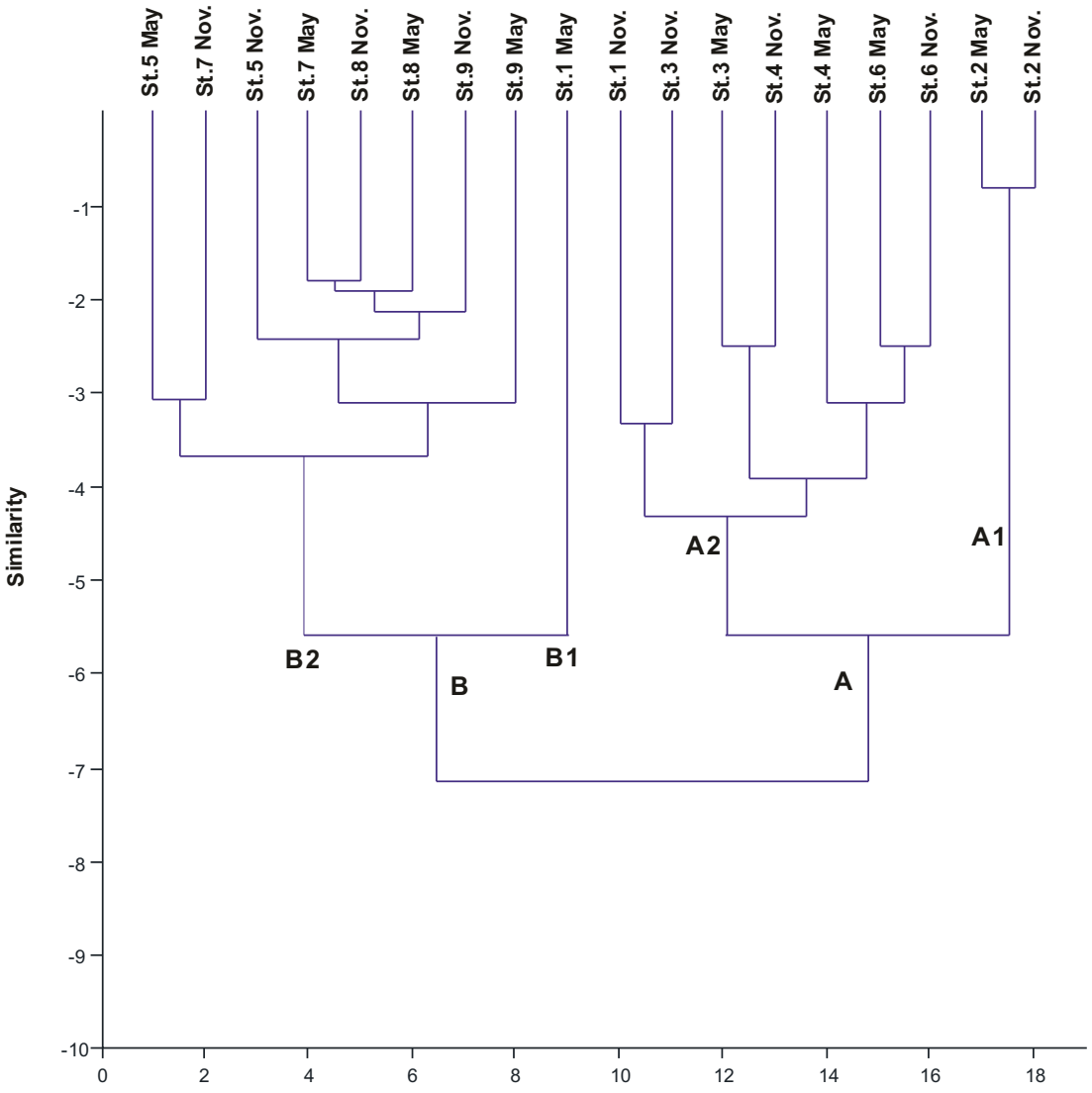

Figure 13. Output of cluster analysis shows the dendrogram classifications of the samples produced by Q-mode cluster analysis using Euclidean distance matrix.

Cluster A (polluted stations) is well characterised in regards to the main features of pollution. It is mainly discriminated by the high values of Pb, Zn, Fe, Cu, Ni, Cr, Cd, Mn, V, Co, and Sc. In addition, it is characterized by high TOC, fine-grained sediments, low DO, and less saline warm turbid water. Cluster A includes sub-clusters (A1) and (A2) according to the different level of pollution and different environmental characters. Sub-cluster (A1) includes the extremely polluted station (St.2 in both seasons) (Fig. 13). This station is mainly dominated by *Ammonia*

tepida, and *Quinqueloculina lata* (Fig. 14). They represent up to 42.4% and 39.3% of the total community, respectively. The foraminiferal assemblage is of low diversity and consists of 26 and 27 species in both seasons. Samples of this sub-cluster appear to be under stressful environmental conditions due to the high pollution level and to the eutrophic and low oxygen conditions. *Ammonia tepida* and *Q. lata* seem to be most resistant to pollution but also to brackish, eutrophic and low oxygen conditions in the study area.

Sub-cluster (A2) comprises comparatively less polluted stations. These stations are located adjacent to pollution sources (St.3, St.4, and St.6). This cluster has an assemblage consisting of *A. tepida, Q. lata, Bolivina variabilis, Rosalina globularis, Porosononion* spp., *Nonion* spp., and *Brizalina* spp. (Figs. 13, 14). The foraminiferal assemblage exhibits higher diversities compared to sub-cluster (A1).

Cluster B consists of 2 subclusters. Station 1 May is represented in sub-cluster (B1). The second sub-cluster (B2) represents relatively clean and/or off-shore stations (St.5, St.7, St.8, and St.9 in both seasons) (Fig. 13). These stations are located apart from the direct influence of pollution. These stations have relatively low concentrations of heavy metals, TOC, and temperature but high dissolved oxygen concentrations, sand content, salinity, transparency, and pH. This cluster is also characterized by having a highly diverse foraminiferal assemblage which indicates a return to nearly normal environmental conditions. The assemblage in this sub-cluster is dominated by *Miliolinella semicostata, Peneroplis pertusus, Quinqueloculina* spp*., Quinqueloculina stelligera, Triloculina trigonula, Ammonia beccarii, Asterigerinata mamilla, Rosalina macropora, Elphidium* spp*.,* and *Quinqueloculina vulgaris* (Figs. 13, 14).

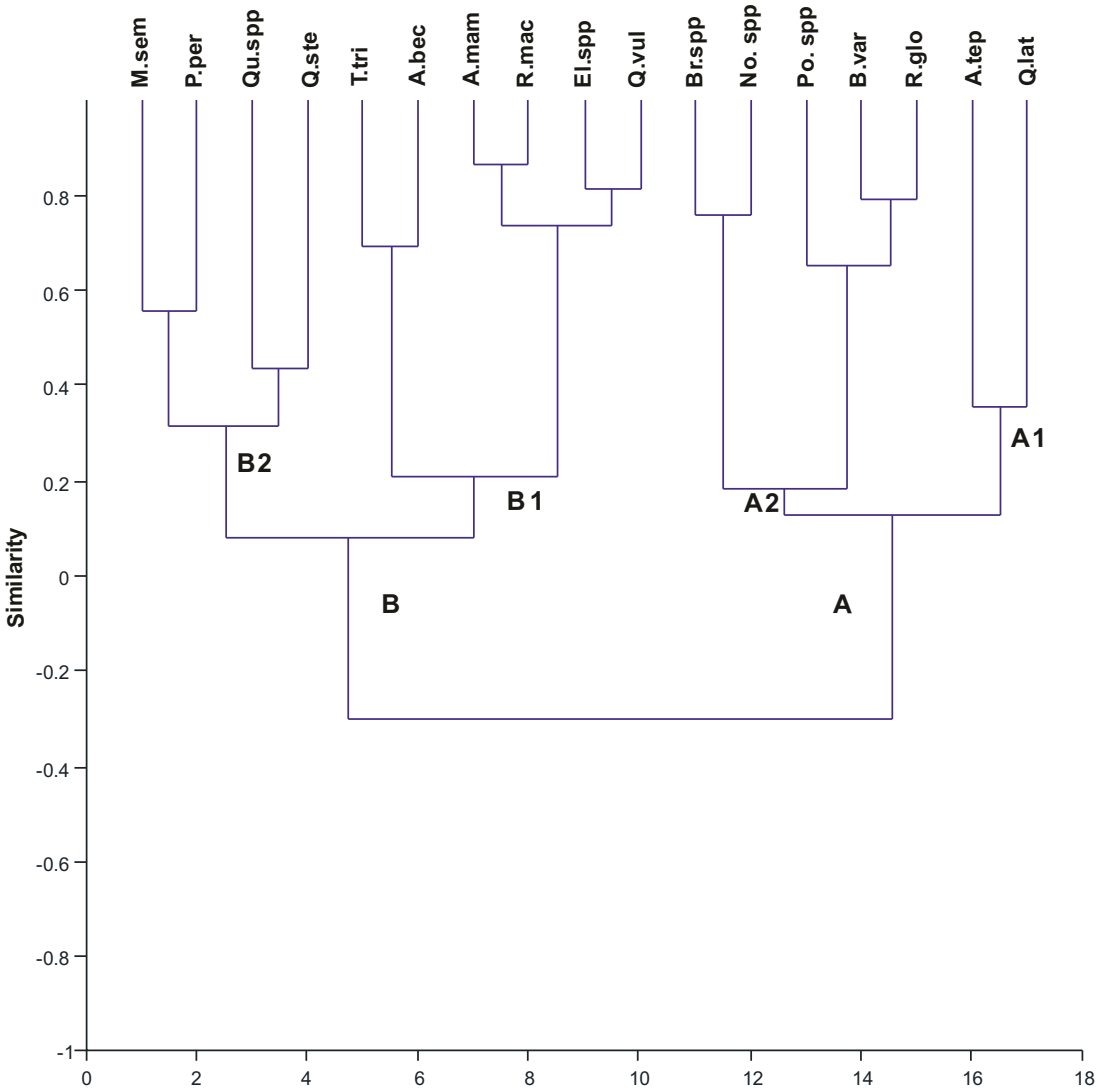

Figure 14. Output of cluster analysis shows the dendrogram classifications of the species produced by R-mode cluster analysis using correlation coefficient matrix. Abbreviations: A.bec: *Ammonia beccarii*, A.tep: *Ammonia tepida*, A.mam: *Asterigerinata mamilla*, B.var: *Bolivina variabilis*, Br.spp: *Brizalina* spp., El.spp: *Elphidium* spp., M.sem: *Miliolinella semicostata*, No.spp: *Nonion* spp., P.per: *Peneroplis pertusus*, Po.spp: *Porosononion* spp., Q.lat: *Quinqueloculina lata*, Qu.spp: *Quinqueloculina* spp., Q.ste: *Quinqueloculina stelligera*, Q.vul: *Quinqueloculina vulgaris*, R.glo: *Rosalina globularis*, R.mac: *Rosalina macropora*, T.tri: *Triloculina trigonula.*

3.4.3.2 Canonical Correspondances Analysis (CCA)

Detrended Correspondence Analysis (DCA) revealed a length of the first gradient of 2.47 standard deviation units (SD), indicating a unimodal foraminiferal distribution

in the study area. Based on this result, the data set was then evaluated with Canonical Correspondance Analysis (CCA).

The first CCA axis explains 46.3% of the variation in the data set, whereas the second CCA axis accounts for 1.6% (Appendix 3). Higher axes were not considered as they explain less than 2% of the total variance. The parameters TOC, DO, sand/silt content, Cd, Zn, Ni, Cu, Fe, Pb, Sc, V, and Co correspond to more than 20% of variance in the dataset (Table 6). Chromium (Cr), transparency, temperature, pH, and salinity are less important, since they correspond to lower variance (Table 6). Therefore, we will focus in the present study on the foraminiferal relation with heavy metal pollution rather than low variance variables. Variance in carbonate content, clay percentage, and Mn concentrations do not relate significantly to foraminiferal distribution. Table 6 shows the importance of different environmental parameters in descending order.

Table 6. Results of the forward selection analysis showing the ranking of environmental parameters according to their variance. Bold variables are significant.

Variable	Variance
TOC	0.30
DO	0.29
sand	0.28
silt	0.28
Cd	0.28
Zn	0.27
Ni	0.26
Cu	0.27
Fe	0.22
Pb	0.21
Sc	0.21
V	0.21
Co	0.21
Cr	0.19
Transparency	0.18
Temperature	0.15
pH	0.11
Salinity	0.10
$CaCO_3$	0.09
clay	0.07
Mn	0.06

Axis 1 separates the stations based on pollution level into two groups (Fig. 15A). The first group is located on the negative side of axis 1. It includes the polluted stations (St.2, St.3, St.4, St.6 in both seasons, and St.1 in Nov.) and their characteristic species. The second group is located on the positive side and consists of the less polluted stations (St.5, St.7, St.8, and St.9) and their characteristic species. The most polluted samples (St.2) in two seasons form a cluster on the extreme negative side of the first axis. A further cluster contains samples from other polluted stations (St.3, St.4, St.6 in both seasons, and St.1 Nov.). Station 1 May lies in-between the two groups. Samples from less polluted sites are randomly spread on the positive side of the first axis (St.5, St.7, St.8, and St.9 in both seasons).

Axis 1 separates species based on heavy metal concentration and different environmental factors (Fig. 15B). Hence, many foraminiferal species are located in the right corner where heavy metal concentrations, TOC, and temperature are lowest, whereas grain size, salinity, DO, transparency, and pH are highest. These species include mainly *Quinqueloculina vulgaris*, *Elphidium* spp., *Asterigerinata mamilla*, *Rosalina macropora*, *Ammonia beccarii*, *Triloculina trigonula*, *Peneroplis Pertusus, Quinqueloculina* spp. and to a lesser extent *Miliolinella semicostata* and *Quinqueloculina stelligera* (Fig. 15B). The species that plot in the left corner are associated with high heavy metal concentrations, temperature, TOC, silt and clay content but low DO, salinity, transparency, and pH. These species include *Ammonia tepida, Quinqueloculina lata, Porosononion* spp., and to a lesser extent *Bolivina. variabilis*, *Nonion* spp., *Brizalina* spp., and *Rosalina globularis* (Fig. 15B). Along the second axis, the pattern of species and samples is less prominent.

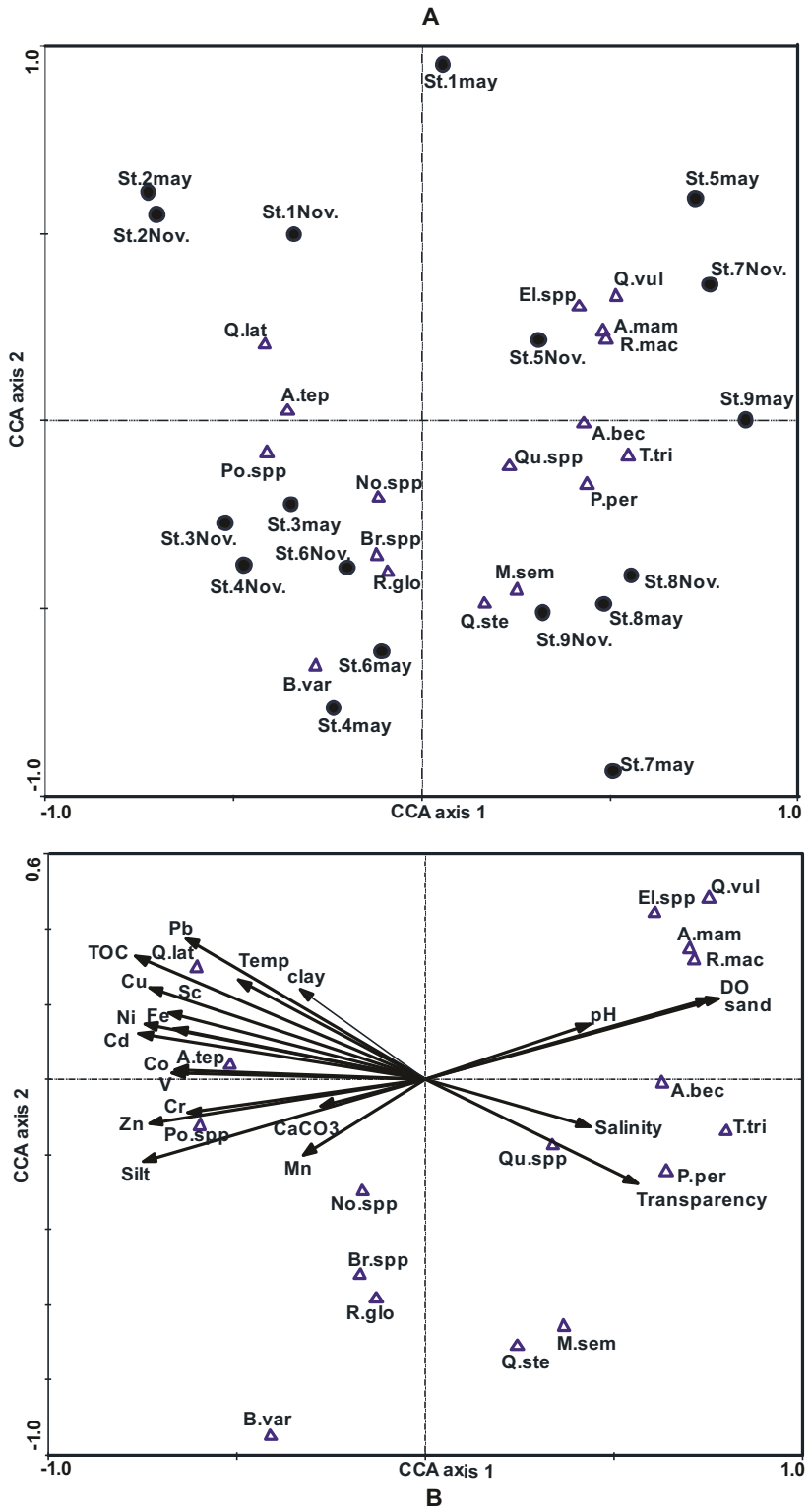

Figure 15. Canonical correspondence analysis (CCA) biplot for relative abundance of foraminiferal species in surface sediment, together with the associated environmental variables. Station scores of 18 samples (A). Species scores of 17 foraminiferal taxa (B). Environmental vectors were omitted from (A) for clarity, but would be arranged as in (B). For species abbreviations see Fig.14.

3.4.4 Test deformations and preservation

Among the total of 105 identified species, 12 species show morphological abnormalities (Table 7). *Quinqueloculina* spp. shows the highest percentage of deformed specimens (22.86%), followed by *Elphidium* spp. (17.14%), *Cibicides refulgens* (14.29%), *A. tepida* (11.43%), and *Porosononion* spp. (8.57%). The deformation in the other species mentioned in Table 7 is limited. Nine morphological deformation types in Abu-Qir Bay are noticeable, comprising abnormal growth (Figs. 5.2, 5.4, 5.8, 5.10, 5.11, 5.13, 5.24, 5.27, 5.28, 5.30), double apertures (Figs. 5.1, 5.3, 5.6, 5.7), abnormal chamber enlargement (Figs. 5.8, 5.17-5.20, 5.25-5.26), loose miliolid coiling (Fig. 5.9), division of the last chamber (Fig. 5.5), discontinuity of growth (Figs. 5.5, 5.21), highly deformed test (Fig. 5.16), aberrant chamber shape and size (e.g., Figs. 5.2, 5.8, 5.12, 5.14, 5.15, 5.18, 5.19, 5.20, 5.23, 5.26, 5.29), and irregular periphery rather than rounded (Figs. 5.15, 5.17, 5.20, 5.22, 5.25, 5.29). Some specimens, e.g., from *Quinqueloculina* spp., exhibit more than one deformation type (division of the last chamber and discontinuity of growth; Fig. 5.5). Maximum percentages of deformed specimens are recorded at St.1 faunas followed by St.2 and St.3 faunas (Table 5). The deformation percentage is higher in cluster A than in cluster B.

The present foraminiferal assemblages show additional evidence for taphonomic processes including microborings, dissolution, and blackening of the tests. The former and the latter are expressed in most of the specimens (e.g., Figs. 5.4, 5.6, 5.14, 5.30, 6.15). Dissolution is well expressed in some genera such as *Peneroplis* and *Elphidium* (Figs. 7.12, 9.15). Microborings are considered as good indicator of bioerosion. These microbioerosional traces include phototrophic boring organisms of cyanobacteria and chlorophytes. In addition, there is some evidence of predation and parasitism of benthic foraminifera on tests of this assemblage (Nielsen et al., 2003).

Table 7. List of all species showing at least one abnormal specimen and the percentage of irregular tests.

Species	Percentage
Ammonia tepida (Cushman, 1926)	11.43
Astrononion stelligerum (d'Orbigny, 1839)	2.86
Cibicides lobatulus (Walker and Jacob, 1798)	5.71
Cibicides refulgens (Montfort, 1808)	14.29
Elphidium advenum (Cushman, 1922)	5.71
Elphidium cf. *crispum* (Linnaeus, 1758)	2.86
Elphidium spp.	17.14
Peneroplis planatus (Fichtel and Moll, 1798)	2.86
Porosononion spp.	8.57
Quinqueloculina spp.	22.86
Sorites orbiculus (Ehrenberg, 1839)	2.86
Spiroloculina spp.	2.86

3.5 Discussion

3.5.1 Ecology and distribution of foraminifera: Natural versus anthropogenic factors

3.5.1.1 Foraminifers as bioindicator of pollution

Based on the variability in foraminiferal assemblages and their relationship with natural and anthropogenic environmental conditions, two distinctive environments are recognized, each with its characteristic faunal community.

Group 1:

This species group appears where surface sediments are characterised by high heavy metal concentrations, rich in TOC, high silt and clay content, and high bottom water temperature, but low DO concentrations, salinity, transparency, and pH. It is characterised by the dominance of *Ammonia tepida, Quinqueloculina lata, Porosononion* spp., and to a lesser extent *Bolivina variabilis, Nonion* spp., *Brizalina* spp., and *Rosalina globularis*.

Species that are abundant in polluted areas are likely to be tolerant (resistant or opportunistic) to the prevalent pollutant. Alternatively, sensitivity to pollution is often expressed through absence of species (Yanko et al., 1999). At lower to mid-latitudes (Mediterranean, Caribbean, and Arabian Seas), *A. tepida* has been reported to dominate in areas close to outfalls of discharged sewage (Seiglie, 1971), chemical and thermal effluents (Seiglie, 1975), fertilizer byproducts (Setty, 1976), caustic soda and chlorine complex (Setty and Nigam, 1984), and heavy metals (Nagy and Alve, 1987; Alve, 1991; Sharifi et al., 1991; Yanko et al., 1994; Samir, 2000; Samir and El-Din, 2001; Armynot du Chatelet et al., 2004; Frontalini and Coccioni, 2008). At Guanabara Bay, Rio de Janeiro, *A. tepida* is dominant and indicates an environment under stress caused by high heavy metal pollution (Vilela et al., 2004). In temperate regions, *Elphidium excavatum* (Terquem) shows particular tolerance to most kinds of contaminants (Bates and Spencer, 1979; Schafer et al., 1991). These observations are in accordance with the results of the present study where *A. tepida* is abundant at polluted stations. We found that living *A. tepida* speciments existed at highly polluted stations (e.g., St.2), confirming its resistance to pollution. Although the heavy metal pollution exerts a major influence on the foraminiferal distribution in the present study, the other environmental parameters have to be also considered. Apart from the pollution level, *A. tepida* is associated with low salinities and organic-rich fine-grained substrate in the semi-closed high turbid basin (St.2). Samir et al. (2003) reported that *A. tepida* is abundant in low energy environments with muddy or sandy surface sediments, characteristic for semi-closed basins. In addition, *A. tepida* is abundant in turbid estuarine environments (Debenay et al., 2006) where it tolerates lower salinities (Walton and Sloan, 1990). Hyams-Kaphzan et al. (2009) reported that *A. tepida* behaves as a dominant element in shelf faunas subject to less extreme disturbances, e.g., oligotrophic sites in the Mediterranean Sea. However, it behaves as an opportunist under disturbed conditions, e.g., at eutrophic sites in the same study area. In eutrophic environments, its dominance is coupled with low total foraminiferal abundances and species richness.

The current study differentiates between the sensitivity of the different types of *Ammonia* to the pollution level. *Ammonia tepida* is considered as pollution-tolerant, while *A. beccarii* is pollution-sensitive. This is obtained from their different affinity towards highly polluted and less polluted stations respectively. This finding is in

agreement with previous results obtained by Poag (1978), and Samir (2000). They reported *A. tepida* as the most tolerant species of the genus *Ammonia*.

Miliolids appear very sensitive to pollution, indicated by the highest percentages of deformed tests in *Quinqueloculina* spp., and the increasing tendency of this group towards unpolluted offshore stations. Cluster analysis and CCA give further confirmation of the sensitivity of this genus (e.g., *Quinqueloculina* spp., *Q. stelligera*, and *Q. vulgaris*). This is in accordance with the results obtained by Rao and Rao (1979) and Samir and El-Din (2001) who reported that Miliolids are less tolerant to pollution. In spite of the pollution sensitivity of *Quinqueloculina* spp., some studies recorded different *Quinqueloculina* species as pollution indicators. In this context, Romano et al. (2008) specified *Quinqueloculina parvula* as a pollution-tolerant species. In the present study in Abu-Qir Bay, *Q. lata* is the only *Quinqueloculina* species which was recognised as pollution indicator species.

The resistance of *B. variabilis* to pollution was confirmed by several studies**.** Armynot du Châtelet (2003) reported that *B. variabilis* is positively correlated to metal pollution (copper, lead and zinc) and organo-tin (TBT, DBT and MBT). The present study also identified *B. variabilis* as a bio-indicator of heavy metal pollution. This species is also abundant in non-polluted sites but adapted to eutrophic anoxic conditions. Most species of the genera *Brizalina* and *Bolivina* are well known to survive in oxygen-deficient environments (Murray, 1991; Rathburn and Corliss, 1994; Bartels-Jónsdóttir et al., 2006; Schumacher et al., 2007). Their flattened elongate morphology (high length/width ratio) is considered to be an adaptation to low oxygen concentrations (Bernhard, 1986). Kaminski et al. (2002) considered *Brizalina* spp. as dysoxic species. In addition, some *Brizalina* species are considered as stress markers, caused by low oxygen conditions (e.g., Rathburn and Corliss, 1994; van Hinsbergen et al., 2005).

In the present study *Nonion* spp. correlates negatively with DO and the sand percentage. This result is confirmed by previous studies that reported a preference of this taxon to muddy and silty sediments (Murray, 1991; Nigam and Chaturvedi, 2000). The study of Panchang et al. (2006) revealed an adaptation of *Nonion* spp. to bottom water oxygenation. In experiments, this taxon migrated to the sediment surface as response to decreasing oxygen levels.

Group 2:

The second group of foraminiferal species mainly includes *Quinqueloculina vulgaris, Elphidium* spp., *Asterigerinata mamilla, Rosalina macropora*, *Ammonia beccarii*, *Triloculina trigonula*, *Peneroplis Pertusus, Quinqueloculina* spp., and to a lesser extent *Miliolinella semicostata and Quinqueloculina stelligera*. This group shows their highest relative abundance at sites characterised by less polluted, coarser sediment with high bottom oxygen concentration, as well as transparent, saline, and cold water.

Highest abundance of *A. mamilla* and *A. beccarii* is related to highest sand contents. Similar preferences are documented in a study from the Adriatic Sea by Jorissen (1987). He reported that *A. mamilla* is restricted mainly to sandy substrates and that *A. beccarii* is related to relatively coarse-grained sediment. Milker et al. (2009) have reported *A. mamilla* from coarse-grained and carbonate rich sediment in cool-water carbonate environments in the Western Mediterranean.

In temperate regions, *E. excavatum* shows particular tolerance to most kinds of contaminants (Bates and Spencer, 1979; Schafer et al., 1991), whereas in our study area, *Elphidium* spp. is recorded at less polluted stations. This could be due to the dominant influence of other environmental parameters, such as DO that is higher at less polluted stations. Commonly, *Elphidium* spp. prefer environments with high oxygen contents (Martins et al., 2006). It is obvious that the foraminiferal distribution is influenced by various environmental variables rather than a single factor. It is noteworthy to mention that *E. excavatum* is not recorded in Abu-Qir Bay.

The distribution of *Quinqueloculina* spp. in Abu-Qir Bay relates to well-oxygenated sediment. This is in accordance with various studies showing that *Quinqueloculina* spp. is the least tolerant taxon to dysoxia and considered as indicator for well oxygenated bottom water (Mullineaux and Lohmann, 1981; Kaiho, 1994; Den Dulk et al., 1998).

The distribution of *P. pertusus* increases in offshore stations, under the influence of low turbulence stations. This species commonly lives as an epiphytic taxon on red soft algae (Leutenegger, 1984; Reiss and Hottinger, 1984) and requires a stable and

low-energy environment with high light intensity (Murray, 1991). Murray (1970) recorded that *Peneroplis* spp. tends to proliferate in warm waters and tolerates hypersaline environments. Al-Zamel and Cherif (1998) noted that the absence of *Peneroplis* spp. in the subtidal area of the western part of Shatt Al-Arab delta was probably due to the influence of the discharge of Shatt Al-Arab rivers. Also, Murray (1991) noted its absence from the north-western part of the Mediterranean Sea and attributed this to the influence of the Rhône discharge.

3.5.1.2 Foraminiferal diversity, density, test deformation and ecosystem stability

Species diversity can be viewed as an important measure of the effect of environmental stress on benthic foraminiferal communities. Accordingly, low species diversity is also characteristic for polluted environments (Yanko et al., 1998; Samir and El-Din, 2001). Changes in species diversity as a response to pollution were discussed by several authors. Schafer (1973) reported that the diversity is reduced close to the effluent sources reflecting adverse environmental conditions in Chaleur Bay, eastern Canada. Bates and Spencer (1979) recorded an increase in foraminiferal diversity and density with increasing distance from the pollution source in Chesapeake Bay. Moreover, Schafer et al. (1991) recognized that species diversity and the total foraminiferal number is much lower in stressed environments. A different foraminiferal response was reported by Alve (1991), Burone et al. (2006), Romano et al. (2008), who found intermediate values of diversity associated with low faunal density at the most polluted sites. Alve and Olsgrad (1999) carried out colonization experiments on foraminifera with increasing copper contamination and found that concentrations up to 2000 mg/kg do not cause a significant decrease in species diversity. In addition, they recorded a significant negative correlation between foraminiferal density and increasing Cu concentration in the sediments. In spite of the high heavy metal concentrations in our study area, the type and magnitude of pollution was obviously not so harmful to the marine benthic environment to cause the complete disappearance of foraminifera, as recorded in other sites strongly polluted by heavy metals (Schafer et al., 1975; Samir, 2000; Elberling et al., 2003; Ferraro et al., 2006). Otherwise, in our study area pollution leads to low-diverse foraminiferal communities. This result is corroborated by data

showing that increased pollution leads to a poor community consisting of few opportunistic species in high numbers (Murray, 1973; Pearson and Rosenberg, 1976), This relation explains the highest values of percentage dominance especially at the heavily polluted St.2.

Apart from the pollution level, other environmental factors also affect foraminiferal diversity. First, the low diversity specifically in St.2 could be explained by the low oxygen content. In the study of Haynes (1981), the presence of low species diversity coincided with constant temperature but stagnant conditions. Loubere (1997) reported that the diversity of foraminifera rises quickly in more oxygenated sediments. Secondly, the increasing diversity in offshore direction could be related to increasing environmental stability coinciding with less stress on the benthic ecosystems (Schafer et al., 1991). Previous studies indicated that in shelf environments the diversity increases with the increase of water depth (El-Halaby, 1993; Al-Zamel and Cherif, 1998). Thirdly, the decreasing diversity in inshore direction could be due to the decreasing salinity as a result of the effluent discharge as is the case in St.3, St.2, St.4, and St.6 in Abu-Qir Bay. Several studies indicated that lower salinities have lead to low diversity, hence the change in salinity affected the density of the water and its osmotic effect influenced the growth of the foraminifera test (Murry, 1973; Al-Haq and Boersma, 1998).

In Abu-Qir Bay the high foraminiferal abundance in polluted stations is related to the coincidence between the high pollution level and high TOC especially at St.2, St.3 and St.6. Various benthic species could benefit from organic wastes as a food source (Watkins, 1961; Yanko et al., 1994). However, heavy metals and chemicals are unlikely to favor any particular species. Bates and Spencer (1979) and Stott et al. (1996) reported increased foraminiferal abundances close to sewage outfalls, where foraminifera benefit directly from increased food resources, or indirectly by reduced predation and competition.

Significant percentages of abnormal tests in the assemblage may be attributed to the environmental stress due to high amplitude ranges of the ecological parameters or extreme environmental conditions (Geslin et al., 1998), such as hypo/hyper salinity, strong changes in dissolved oxygen, temperature, pH, irregularity of substrate, and pollution (Geslin et al., 2000) or due to strong hydrodynamic variability (Bergin et

al., 2006). In Abu-Qir Bay, St.1, St.2, and St.3 are particularly suitable to the study of environmental stress due to high pollution level and because St.1 (harbour) is additionally influenced by a turbulent hydrodynamic environment. As a result, these stations have highest percentages of foraminiferal test deformations. This particularly unstable environment at St.1 also results in a low number of living specimens.

3.5.2 Impact of taphonomic processes on test preservation

Fossil foraminiferal assemblages are the product of foraminiferal production, taphonomic loss, and bioturbation (Berkeley et al., 2007). Taphonomic processes within intertidal environments may modify assemblages with respect to the living community from which they were produced (Cummins et al., 1986). The most important taphonomic processes in intertidal environments are those associated with early diagenesis. While the organic cements of agglutinated taxa may be directly oxidised, the associated changes in the saturation state of pore waters with respect to calcium carbonate may affect calcareous species (Berkeley et al., 2007).

In the present study the genera *Elphidium* and *Peneroplis* show high signs of dissolution. This observation suggests that these genera seem to be less robust to dissolution than the others. Dissolution of calcareous tests has been investigated by many authors. The majority of them attribute this phenomenon principally to the lower values of pH (Boltovskoy and Wright, 1976), temperature (Forti and Röttger, 1967), or the decay of organic matter (Thunell, 1976). Berkeley et al. (2007) reported that the aerobic oxidation of organic matter promotes foraminiferal dissolution due to the production of carbon dioxide, which dissolves resulting in further increasing of the concentration of carbonic acid (Krauskopf and Bird, 1995). In our study area the temperature shows minor seasonal ranges in both seasons (21°C-25.5°C), pH values are consistently within the range of 7.7 to 8.26, with the exception of a low value at St.3 (7.27), while the organic carbon content shows large variations among stations (0.06% - 6.71%). It appears most likely that the organic pollution is associated with foraminiferal dissolution.

We recorded high frequency of pyritized foraminiferal tests in Abu-Qir Bay, which may indicate oxygen-deficient environments. Maiklem (1967) regarded the blackening of foraminiferal tests to sediment-reducing conditions in shallow water.

The reasons of pyritization are not completely clear. Some have suggested that it is connected to chemical processes that result in metabolization of organic matter under anaerobic conditions by sulfate-reducing bacteria, diffusion of sulfide into sediments, or concentration and reactivity of the iron minerals. The process is connected to the redox conditions and the concentration of H_2S (Yanko et al., 1999).

The high percentage of microboring of foraminifera in the study area is considered as good indicator of bioerosion. Bioerosion traces confirm the predation and parasitism of benthic foraminifera which are widespread phenomena in modern and fossil marine environments (Nielsen et al., 2003). Nielsen (1998) suggested that the majority of the traces may be interpreted as evidence of predation of the foraminifera by several unknown predators, including benthic as well as planktonic organisms. A few of the observed traces may be host species-specific, being related to certain foraminifera; for example the trace *Dipatulichnus rotundus* occurs only on the planktonic *Orbulina universa* (d'Orbigny) (Nielsen and Nielsen, 2001).

3.6 Conclusions

In this research, benthic foraminifera allowed for evaluation of the ecological health of the benthic marine coastal environment in Abu-Qir Bay. The results of quantitative analyses on foraminiferal benthic assemblages, processed with geochemical and sedimentological data, helped to characterise the study area by recognising two distinct sectors, with different environmental characters and different degrees of pollution. The first sector, comprising the most polluted stations, is characterised by sediment rich in TOC, high silt and clay content, and high bottom water temperature, but low DO concentrations, salinity, transparency, and pH. The environmental stress in these stations is reflected by low faunal diversities, high percentage dominance, and high deformation rates. This study confirmed that morphological abnormalities can be utilised in pollution assessment. Polluted environments favour the presence of pollution-tolerant species such as *Ammonia tepida, Quinqueloculina lata* and *Porosononion* spp. The second sector, comprising the less polluted stations, is characterised by coarser sediment with high bottom oxygen concentration, transparent, saline, and cold waters. These sites are dominated by pollution-sensetive taxa such as *Quinqueloculina vulgaris, Elphidium* spp.,

Asterigerinata mamilla, Rosalina macropora, *Ammonia beccarii*, *Triloculina trigonula*, *Peneroplis Pertusus,* and *Quinqueloculina* spp.

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Appendix 1. Faunal reference list of the species occurring in more than 95% of one series of samples arranged in alphabetical order.

Ammonia beccarii (Linnaeus, 1758) Fig. 9.5.

Ammonia tepida (Cushman, 1926) Fig. 9.7.

Asterigerinata mamilla (Williamson, 1858) Figs. 8.18, 9.1.

Bolivina variabilis (Williamson, 1858) Figs. 7.16-7.17.

Brizalina spp.

Elphidium spp.

Miliolinella semicostata (Wiesner, 1923) Figs. 7.2-7.3.

Nonion spp.

Peneroplis pertusus (Forskäl, 1775) Fig. 7.10.

Porosononion spp. Figs. 9.16-9.17.

Quinqueloculina lata (Terquem, 1876) Fig. 6.17.

Quinqueloculina spp.

Quinqueloculina stelligera (Schlumberger, 1893) Fig. 6.19.

Quinqueloculina vulgaris (d'Orbigny, 1826) Fig. 6.21.

Rosalina globularis (d'Orbigny, 1826).

Rosalina macropora (Hofker, 1951) Fig. 8.9.

Triloculina trigonula (Lamarck, 1804) Fig. 7.7.

Appendix 2. Relative abundance of all species collected in the two sampling periods.

Species	St.1 May	St.1 Nov.	St.2 May	St.2 Nov.	St.3 May	St.3 Nov.	St.4 May	St.4 Nov.	St.5 May
Abditodentrix rhomboidalis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adelosina carinata-striata	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adelosina duthiersi	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0
Adelosina intricata	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
Adelosina mediterranensis	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.4
Adelosina putchella	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Adelosina spp.	0.0	0.4	0.0	0.0	1.0	0.0	0.0	0.4	0.4
Agglutinated form	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Ammonia beccarii	2.9	0.0	0.5	0.0	6.0	0.4	5.0	0.0	4.2
Ammonia gaimardi	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ammonia parkinsoniana	0.2	0.9	1.2	0.4	3.3	0.0	1.8	3.5	0.8
Ammonia spp.	0.4	0.0	0.0	0.4	3.7	0.4	0.0	1.7	0.4
Ammonia tepida	10.0	33.3	41.7	42.4	33.8	26.4	27.5	30.3	0.8
Amphistegina spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Articulina mucronata	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.4
Articulina spp.	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
Asterigerinata mamilla	18.7	4.3	0.9	0.6	1.7	0.0	0.5	0.9	17.6
Astrononion stelligerum	0.2	0.0	0.2	0.0	0.7	0.0	0.0	0.0	0.4
Bolivina variabilis	0.0	0.0	0.0	0.2	0.3	0.4	5.5	0.4	0.0
Brizalina difformis	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Brizalina dilatata	0.0	0.0	0.0	0.0	0.3	0.0	0.9	1.3	0.0
Brizalina spathulata	0.0	1.3	0.0	0.4	0.0	0.7	1.4	4.3	0.0
Brizalina spp.	0.7	0.0	0.2	0.4	5.4	9.4	2.3	10.0	0.4
Brizalina striatula	0.0	0.0	0.2	0.9	2.0	2.5	2.3	1.3	0.0
Buccella granulata	0.2	0.4	0.2	0.0	0.0	0.7	0.0	0.0	1.3
Bulimina elongata	0.0	0.9	0.0	0.2	0.0	0.0	0.5	0.0	0.0
Bulimina marginata	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bulimina spp.	0.0	0.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Cassidulina crassa	0.2	0.0	0.0	0.0	0.3	1.8	0.0	0.0	0.0
Cassidulina spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
Cibicidella variablis	0.0	0.0	0.0	0.0	0.0	0.4	0.5	0.0	0.0
Cibicides lobatulus	0.2	0.4	0.0	0.0	1.3	0.0	2.3	3.5	2.1
Cibicides refulgens	8.0	1.7	2.1	0.4	0.0	1.8	0.0	0.0	5.0
Cibicides spp.	0.4	0.0	0.0	0.0	0.7	0.0	1.4	0.9	0.4
Cibicidoides spp.	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0
Cornuspira spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Discorbinella bertheloti	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Elphidium jenseni	2.4	1.3	0.2	0.6	0.3	1.8	0.0	0.9	0.8
Elphidium aculeatum	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.4
Elphidium advenum	0.4	0.0	1.2	0.4	1.3	0.4	2.3	0.0	4.6
Elphidium complanatum	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Elphidium crispum	4.7	0.0	0.5	0.0	0.0	0.4	0.5	0.0	2.1
Elphidium spp.	15.6	5.5	0.9	1.3	0.3	0.8	0.9	2.6	9.6
Elphidium translucens	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Eponides concamerata	0.4	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
Fissurina orbignyana	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fissurina spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Floresina spp.	0.0	0.9	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Fursenkoina spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
Gavelinopsis praegeri	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gavelinopsis transluccus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glabratella hexacamerata	0.0	0.4	0.0	0.0	0.0	0.0	0.9	0.0	0.0

Glabratella erecta	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
Glabratella spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hyalinea baltica	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lachlanella spp.	0.2	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0
Lachlanella variolata	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lenticulina spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Miliolinella circularis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
Miliolinella labiosa	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
Miliolinella semicostata	0.2	0.4	0.5	0.0	1.3	0.0	1.4	0.0	0.0
Miliolinella spp.	0.0	1.3	0.0	0.0	0.6	1.1	0.0	1.3	0.4
Miliolinella subrotunda	0.0	0.9	0.0	0.0	0.0	2.2	0.9	0.0	0.0
Neoconorbina terquemi	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
Nonion commune	0.0	0.0	0.0	0.2	0.0	0.0	0.5	0.0	0.0
Nonion spp.	1.1	0.9	0.2	0.2	2.0	2.9	0.0	8.7	2.1
Patellina corrugata	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Peneroplis pertusus	2.7	1.3	0.0	0.0	1.0	0.0	0.5	0.0	3.8
Peneroplis planatus	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.4
Planorbulina mediterranensis	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
Porosononion spp.	0.2	0.0	4.4	3.1	4.0	5.4	1.4	3.5	0.0
Pyrgo spp.	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Quinqueloculina auberiana	0.9	0.4	0.0	0.2	0.0	0.0	0.0	0.0	0.0
Quinqueloculina laevigata	0.4	0.4	0.0	0.0	0.0	1.8	1.4	0.9	0.0
Quinqueloculina lata	0.0	23.9	38.2	39.3	4.7	19.1	5.0	7.4	4.2
Quinqueloculina parvula	0.2	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
Quinqueloculina seminula	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Quinqueloculina stelligera	0.2	0.9	0.0	0.0	0.7	0.0	4.6	1.3	0.0
Quinqueloculina viennensis	1.6	1.7	0.0	0.0	0.3	1.1	1.4	2.6	0.0
Quinqueloculina vulgaris	7.1	0.9	0.7	0.0	0.3	0.0	0.0	0.0	2.5
Quinqueloculina neapolitana	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0
Quinqueloculina spp.	5.6	9.4	2.1	5.0	5.4	7.2	12.4	3.5	18.0
Rosalina bradyi	0.7	0.0	0.5	0.2	0.0	2.2	0.0	0.0	0.0
Rosalina globularis	0.0	0.9	0.0	0.9	0.7	5.8	4.1	1,3	0.0
Rosalina macropora	5.1	2.1	0.2	0.6	0.3	0.0	0.0	0.0	7.5
Rosalina spp.	0.2	0.0	0.0	0.7	1.7	0.7	4.1	1.7	2.1
Sigmoilinita costata	0.7	0.0	0.0	0.2	0.3	0.4	0.9	0.0	1.3
Siphonaperta irregularis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Siphonaperta spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Siphouvigerina spp.	0.0	0.0	0.0	0.0	0.3	0.4	0.5	0.0	0.0
Sorites orbiculus	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Spirillina spp.	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Spiroloculina dilatata	2.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1
Spiroloculina excavata	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Spiroloculina rotundata	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Spiroloculina nummiformis	0.0	0.0	0.0	0.0	0.0	0.0	0,0	0.0	1.1
Spiroloculina spp.	1.1	0.3	0.0	0.7	0.7	0.4	0.0	0.4	1.3
Spiroloculina tenuiseptata	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Triloculina laevigata	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.0	0.0
Triloculina spp.	0.7	0.0	0.2	0.0	0.0	0.0	0.0	0.9	0.4
Triloculina tricarinata	0.2	0.0	0.0	0.0	0.3	0.0	0.5	0.4	1.3
Triloculina trigonula	0.2	0.4	0.5	0.0	0.0	1.1	0.0	0.0	0.0
Verneuilina spp.	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Vertebralina striata	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix 2 (cont.)

Species	St.5 Nov.	St.6 May	St.6 Nov.	St.7 May	St.7 Nov.	St.8 May	St.8 Nov.	St.9 May	St.9 Nov.
Abditodentrix rhomboidalis	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adelosina carinata-striata	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
Adelosina duthiersi	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
Adelosina intricata	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
Adelosina mediterranensis	1.0	1.7	0.0	0.0	1.4	0.0	1.4	0.9	0.4
Adelosina putchella	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adelosina spp.	1.0	0.4	0.9	4.5	3.2	0.0	1.4	4.4	3.0

Agglutinated form	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ammonia beccarii	3.5	0.0	0.9	0.6	8.3	3.0	3.2	11.3	1.5
Ammonia gaimardi	0.0	0.0	0.0	0.0	0.0	1.2	0.0	0.6	0.0
Ammonia parkinsoniana	3.5	2.1	2.2	0.6	0.0	0.0	0.0	0.0	0.8
Ammonia spp.	0.5	2.1	0.4	0.0	3.2	0.0	0.0	0.3	0.0
Ammonia tepida	8.0	15.8	20.0	0.6	0.9	1.2	1.8	0.9	5.7
Amphistegina spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
Articulina mucronata	0.5	1.3	0.0	1.1	0.0	0.0	0.0	0.0	0.0
Articulina spp.	0.5	0.0	0.9	1.1	0.0	0.0	0.0	0.0	0.0
Asterigerinata mamilla	9.5	2.1	1.3	6.8	6.0	4.2	3.6	6.0	3.4
Astrononion stelligerum	0.0	0.8	0.9	0.0	0.5	0.0	0.5	0.6	0.0
Bolivina variabilis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Brizalina difformis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Brizalina dilatata	0.0	0.0	0.4	0.0	0.0	0.6	0.0	0.0	0.8
Brizalina spathulata	0.0	2.1	0.0	0.0	0.0	0.6	0.0	0.0	0.0
Brizalina spp.	2.0	2.9	3.5	4.0	0.9	3.6	1.4	0.0	2.3
Brizalina striatula	0.5	1.7	0.4	0.0	0.0	0.0	0.0	0.0	0.8
Buccella granulata	2.0	0.0	0.4	2.3	2.3	1.8	3.2	1.3	1.1
Bulimina elongata	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bulimina marginata	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bulimina spp.	0.0	0.4	1.7	0.0	0.0	0.0	0.5	0.0	1.1
Cassidulina crassa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cassidulina spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cibicidella variablis	0.0	0.0	1.3	0.6	0.0	0.0	0.5	0.0	0.0
Cibicides lobatulus	2.0	2.5	0.0	0.0	0.9	0.6	2.7	0.3	1.1
Cibicides refulgens	3.5	0.0	2.2	1.1	6.9	2.4	3.6	4.4	3.0
Cibicides spp.	0.0	0.0	0.0	0.6	2.3	0.0	0.0	0.6	0.8
Cibicidoides spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cornuspira spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Discorbinella bertheloti	0.5	0.0	0.0	0.6	0.5	1.8	0.0	0.0	0.4
Elphidium jenseni	0.0	0.0	0.4	0.0	0.0	0.0	0.5	0.3	0.0
Elphidium aculeatum	0.0	0.0	0.0	0.0	1.4	0.0	0.0	2.8	0.0
Elphidium advenum	4.0	0.8	2.2	2.8	2.8	3.6	3.2	0.9	2.3
Elphidium complanatum	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.9	0.0
Elphidium crispum	0.0	0.4	0.0	0.0	0.0	2.4	0,9	0.3	0.0
Elphidium spp.	6.0	1.6	1.7	1.7	11.9	1.2	2.7	5.1	1.9
Elphidium translucens	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Eponides concamerata	1.0	0.4	0.4	0.6	0.0	1.2	0.5	0.3	0.0
Fissurina orbignyana	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fissurina spp.	0.5	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0
Floresina spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.4
Fursenkoina spp.	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
Gavelinopsis praegeri	0.0	1.3	0.0	0.6	0.0	0.0	0.0	0.3	0.8
Gavelinopsis transluccus	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.4
Glabratella hexacamerata	0.0	1.3	1.7	2.8	0.9	0.0	1.4	0.9	0.8
Glabratella erecta	0.5	0.0	0.0	1.1	0.5	1.8	2.7	0.0	0.8
Glabratella spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.9	1.6	0.0
Hyalinea baltica	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0
Lachlanella spp.	0.0	0.0	0.0	0.0	0.0	0.6	1.8	0.3	0.0
Lachlanella variolata	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Lenticulina spp.	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.3	0.0
Miliolinella circularis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Miliolinella labiosa	0.0	0.0	0.0	0.6	0.0	0.0	0.9	0.0	0.8
Miliolinella semicostata	0.5	0.8	0.9	3.4	0.0	1.2	1.4	1.3	5.3
Miliolinella spp.	1.0	2.5	2.7	5.7	0.0	4.8	4.1	1.8	1.9
Miliolinella subrotunda	1.0	0.8	0.9	2.3	0.0	0.0	2.7	0.0	1.1
Neoconorbina terquemi	0.0	0.8	0.0	0.6	0.0	1.2	0.9	1.3	0.4
Nonion commune	0.0	1.3	0.0	0.0	0.0	0.6	0.0	0.0	0.0
Nonion spp.	0.0	0.8	1.7	0.0	0.0	3.0	0.0	0.0	1.9
Patellina corrugata	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
Peneroplis pertusus	2.0	2.5	2.2	3.4	2.3	7.3	2.7	5.7	6.4
Peneroplis planatus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0
Planorbulina mediterranensis	1.5	0.4	0.0	0.0	0.0	0.0	1.8	0.9	0.4
Porosononion spp.	0.5	0.8	3.9	0.0	0.0	0.0	0.9	0.0	0.0
Pyrgo spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.3	0.0
Quinqueloculina auberiana	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0
Quinqueloculina laevigata	2.5	0.0	1.3	1.1	3.7	3.0	2.7	0.9	1.9

Quinqueloculina lata	5.0	3.3	10.4	1.1	0.0	3.0	1.4	0.6	6.1
Quinqueloculina parvula	1.0	0.0	1.3	4.0	0.0	0.6	2.3	0.3	0.8
Quinqueloculina seminula	0.0	0.4	0.0	0.6	0.0	0.0	0.0	0.0	0.8
Quinqueloculina stelligera	2.0	6.7	1.7	4.5	0.9	4.8	2.3	0.3	1.9
Quinqueloculina viennensis	2.5	4.2	0.9	2.8	0.5	1.2	1.4	1.9	2.7
Quinqueloculina vulgaris	1.5	0.4	0.4	0.0	4.6	3.6	1.4	0.6	0.4
Quinqueloculina neapolitana	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Quinqueloculina spp.	11.6	17.5	11.3	11.4	19.7	10.9	10.4	8.2	14.0
Rosalina bradyi	0.0	1.7	0.9	2.3	0.0	1.2	1.4	0.6	0.4
Rosalina globularis	2.0	2.9	5.2	2.8	0.0	0.6	2.3	0.0	1.5
Rosalina macropora	2.0	1.3	0.4	0.6	3.2	2.4	1.4	3.5	3.4
Rosalina spp.	4.5	5.0	3.9	1.7	2.8	3.6	4.1	0.6	3.4
Sigmoilinita costata	2,0	1.7	2.6	0.6	0.9	3.0	1.4	2.8	2.7
Siphonaperta irregularis	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Siphonaperta spp.	0.0	0.0	0.0	1.1	0.0	0.0	0.5	0.0	0.0
Siphouvigerina spp.	0.0	0.0	0.4	1.7	0.0	0.0	0.9	0.0	0.0
Sorites orbiculus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
Spirillina spp.	0.0	0.0	0.5	0,0	0.0	0.0	0.0	0.0	0.0
Spiroloculina dilatata	0.2	0.0	0.0	0.0	0.2	0.5	0.0	1.0	0.0
Spiroloculina excavata	0.0	0.0	0.0	0.6	0.2	0.6	0.0	0.3	0.0
Spiroloculina rotundata	0.1	0.0	0.0	0.2	0.1	0.7	0.5	0.4	0.1
Spiroloculina nummiformis	0.2	0.0	0.0	0.2	0.1	0.3	1.0	1.0	0.2
Spiroloculina spp.	1.0	0.0	0.4	3.0	0.7	1.5	0.8	2.0	0.5
Spiroloculina tenuiseptata	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.4
Triloculina laevigata	0.5	0.4	0.0	0.6	0.0	0.0	0.9	0.0	0.4
Triloculina spp.	0.5	0.0	0.4	4.5	0.0	0.6	2.3	0.3	2.3
Triloculina tricarinata	0.0	0.0	0.4	0.6	1.8	3.6	0.5	1.3	1.1
Triloculina trigonula	1.0	0.4	0.4	0.6	3.7	0.6	4.5	8.5	2.3
Verneuilina spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Vertebralina striata	0.0	0.0	0.0	2.3	0.0	0.6	0.0	2.8	0.0

Appendix 3. Eigenvalues of the first two CCA axes, and direction of foraminiferal species and stations along the first two CCA axes. For abbreviations see Figure 14.

Eigenvalue CCA1 axis 1: 46.3%			**Eigenvalue CCA1 axis 2: 1.6%**		
Species	**Axis 1**	**Axis 2**	**Stations**	**Axis 1**	**Axis 2**
A.bec	0.80	-0.01	St.1 May	1.21	1.76
A.tep	-0.66	0.05	St.1 Nov.	-0.63	0.92
A.mam	0.89	0.44	St.2 May	-1.34	1.12
B.var	-0.53	-1.21	St.2 Nov.	-1.32	1.03
Br.spp	-0.22	-0.67	St.3 May	-0.63	-0.42
El.spp	0.77	0.56	St.3 Nov.	-0.98	-0.49
M.sem	0.47	-0.84	St.4 May	-0.44	-1.42
No.sp	-0.21	-0.38	St.4 Nov.	-0.88	-0.71
P.per	0.81	-0.31	St.5 May	1.29	1.17
Po.spp	-0.77	-0.16	St.5 Nov.	0.64	0.31
Q.lat	-0.78	0.38	St.6 May	0.05	-1.28
Qu.spp	0.43	-0.22	St.6 Nov.	-0.47	-0.60

Q.ste	0.31	-0.91	St.7 May	0.88	-1.66
Q.vul	0.96	0.61	St.7 Nov.	1.45	0.63
R.glo	-0.17	-0.75	St.8 May	0.91	-0.92
R.mac	0.91	0.41	St.8 Nov.	1.01	-0.75
T.tri	1.01	-0.18	St.9 May	1.59	0.01
			St.9 Nov.	0.59	-0.95

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Chapter 4

80 years trophic history of the distal part of the Po-river discharge plume: Evidence from a high temporal resolution dinoflagellate cyst record

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Abstract

To obtain insight in the trophic history of the distal part of the Po-river discharge plume a high temporal resolution dinoflagelate cyst record of the last 80 years has been established. Changes in total organic carbon concentration of the sediment, cyst production rates, and the cyst association have been compared with information about local air temperature and river discharge rates during the last 80 years as well as with nitrate/ammonia/phosphate concentrations of Po-discharge waters and total Italian fertilizer use of the last 36 and 46 years respectively. We show that changes in dinoflagellate cyst production are strongly positively related to Po-river discharge rates throughout the study interval indicating fluctuations in quantity of discharge-plume waters into the gulf. Changes in the dinoflagellate cyst association composition provide insight in the water-quality of the inflow waters. An increase in relative abundances of the species *Lingulodinium machaerophorum* which nowadays is characteristic for nutrient enriched waters in estuarine environments, indicates a change in water quality already as early as 1932. From 1955 – 1970 we reconstruct a strong change in water quality towards more eutrophic conditions based on the strong increase in accumulation rates of species that are characteristic for eutrophic environments such as *Echinidinium* spp*., Lingulodinium machaerophorum,* and *Stelladinium stellatum* and to a lesser extent *Brigantedinium* spp., *Polykrikos schwarzyii, Quincususpis concreta,* and *Lejeunecysta sabrina.* This increase corresponds to enhanced fertilizer use in Italy and first occurrence of eutrophication related anoxic events in the North Adriatic Sea.

From 1980 onward we observe a stabilisation of TOC concentrations, cyst accumulation rates, and association composition followed by a decrease in relative abundances of the nutrient indicators *L. machaerophorum* and *P. schwarzyii* suggesting a slight improvement of the water quality. We speculate this improvement to be related to a reduction in phosphate concentrations in river discharge waters as a result of governmental measurements.

Keywords: dinoflagellate cyst; eutrophication; high resolution; Po-river.

4.1 Introduction

Today eutrophication is an increasing environmental and economic problem in numerous coastal areas. Human activity has accelerated nutrient and trace element enrichment in these regions during the last century with increased inputs of mostly nitrogen and phosphorus, stimulating production of phytoplankton. Although in an early stage, eutrophication can have a positive impact on the planktic and benthic communities by increasing the nutrient availability and food supply, successive increases in nutrients and/or trace-element loads can lead to undesirable changes in the ecosystem such as a decrease in species diversity, over-production and algal blooms that, in turn, can result in oxygen depletion (e.g. Pearson and Rosenberg, 1978; Cooper and Brush, 1993; Justic et al., 1995; Nixon, 1995; Rabelais et al., 1996; Sullivan, 1999; Arai, 2001; Boesch et al., 2001; Cloern, 2001). In the last decades the awareness of the problems caused by eutrophication and its social-economic effects increased and many national and local governments started active waste-management to improve the coastal water quality. One of the main questions of these governments is to estimate in how far and in which form their activities have had effect on the coastal ecosystems.

One of the coastal regions in Europe that suffers most from eutrophication is the region influenced by Po-river discharge water. The Po-river is the largest Italian river which catchment basin receives input from the southern part of Alps in the north and west and from the Appenine mountain range in the south. The Po is used as a source of water as well as being a receiver of waste passing through the countries most intensively industrialized regions and used by agriculture. Increasing anthropogenic activity in the region since the beginning of the last century is assumed to have resulted in a steady increasing nutrient and trace element load of the river water that, in turn could be related to e.g. the more frequent occurrences of (toxic) plankton blooms and anoxia in the North Adriatic Sea (e.g. Giordani and Angiolini, 1983; Chiaudani et al., 1983; Justic, 1987; Marchetti et al., 1988; Degobbis, 1989; Boni et al., 2000). Since about the 1980's a trend of reduced concentrations of phosphate and ammonia can be observed which results in a decrease in upper chlorophyll-*a* concentration of the Northern Adriatic Sea during the last decade (de Wit and Bendoricchio, 2001; Solidoro et al., 2009; Mozetiç et al., 2010). It is suggested that

these observations could be the result of both climate factors (e.g. climatically induced reduced river outflow) and anthropogenic activities (cultural oligotrophication; see discussion in Mozetiç et al., 2010). It is however unclear in how far which factors might be responsible for the observed changes.

A way to obtain more insight into this problematic issue is to study long-term trends in plankton association changes on a high temporal resolution and compare this with both changes in climate and anthropogenic activities in the region. This can be achieved by studying fossil dinoflagellate cyst assemblages in well-dated bottom sediments. Dinoflagellates are known to react very sensitive in population growth and association composition of changes in environmental conditions in upper waters (e.g. Evgenidou et al., 1999). High nitrogen loading, accompanied by other factors, such as water column stratification and high allochthonous organic matter loading, may lead to nuisance blooms of (toxic) dinoflagellate species (e.g. Paerl, 1988; Smayda, 1989, 1990; Anderson et al., 2002). A considerable part of dinoflagellates are able to produce species-specific cysts that have a high preservation potential. These cysts have been proven useful as eutrophication indicators in marine settings, especially in estuarine and fjord systems (e.g. Dale and Fjellsa˚, 1994; Saetre et al., 1997; Thorsen and Dale, 1997; Dale et al., 1999; Matsuoka, 1999; Dale, 2001; Matsuoka 2001; Pospelova et al., 2002, 2004, 2005; Sangiorgi and Donders, 2004; Dale 2009; Shin et al., 2010)

Here we document the results of a dinoflagellate cyst study where we reconstruct in detail the trophic history of the last 80 years of the coastal region of the eastern part of the Golfo di Taranto using the detailed information that is present about the modern day distribution of dinoflagellate cysts in the marine surface sediments of the region (Nichetto et al., 1995; Belmonte et al., 1998; Rubino et al., 2000; Sangiorgi et al., 2001; Zonneveld et al, 2009). The study site is situated at the distal part of the Po-river discharge plume. This plume can be traced as a coastal band of high nutrient sediment loaded water mass from its source in the northern Adriatic Sea throughout the complete Adriatic Sea, the Strait of Otranto into the Golfo di Taranto. It has been shown that the Po-river is the main contributor of water and sediment load to this plume despite the fact that on its way to the south the plume waters are additionally spiced by discharge of, relative to the Po-river, small eastern Italian rivers. However,

in comparison to the Po-river, discharge of nutrient and trace elements of these rivers form only a minor part (e.g. Matteucci and Frascari, 1997; Penna et al., 2004; Milligan and Cattaneo, 2007).

We show that cyst accumulation rates can be related to Po-river discharge variability whereas changes in association and accumulation rates of individual species can be related to anthropogenic activity in the Po-river catchment area. We suggest that variability in accumulation rates of these species can be used to reconstruct the quality of discharge-water rather than the quantity. We discuss the value of these findings for future eutrophication studies.

4.2 Oceanographic setting

The Golfo di Taranto is a small sub-basin basin of the Eastern Mediterranean Sea that is land-locked on three sides by the southern part of Italy. Its hydrography is closely linked to the ocean circulation of both the Adriatic Sea as well as the Ionian Sea. The Adriatic Sea in turn is another side basin of the Eastern Mediterranean Sea oriented NW/SE direction (Fig. 1). It is land-locked by Italy in the west and Balkan countries in the east. It can be divided into two basins of maximal 270 m and 1250 m depths which are separated by a sill of about 200 m water depth. It is connected in the south to the Eastern Mediterranean Sea by the Strait of Otranto with a sill at 750 m depth. The surface waters of the northern basin are strongly influenced by Po-river discharge water. The Po-river is the largest Italian river draining the southern part of the Alps and northern part of Italy. It enters the north-western North Adriatic Sea through a deltaic system. The discharge waters that enter the marine realm have a clear physical and biological character that can be traced along the eastern Italian margin southward throughout the entire Adriatic Sea as the so called ”Po-discharge plume”. Along the way, the plume waters are additionally spiced by sediment loaded, fresh, nutrient and element rich waters from local eastern Italian rivers. However, the loads of nutrients and sediments of these local rivers are considerably lower with respect to the Po-river discharge (e.g. Penna et al., 2004; Milligan and Cattaneo, 2007).

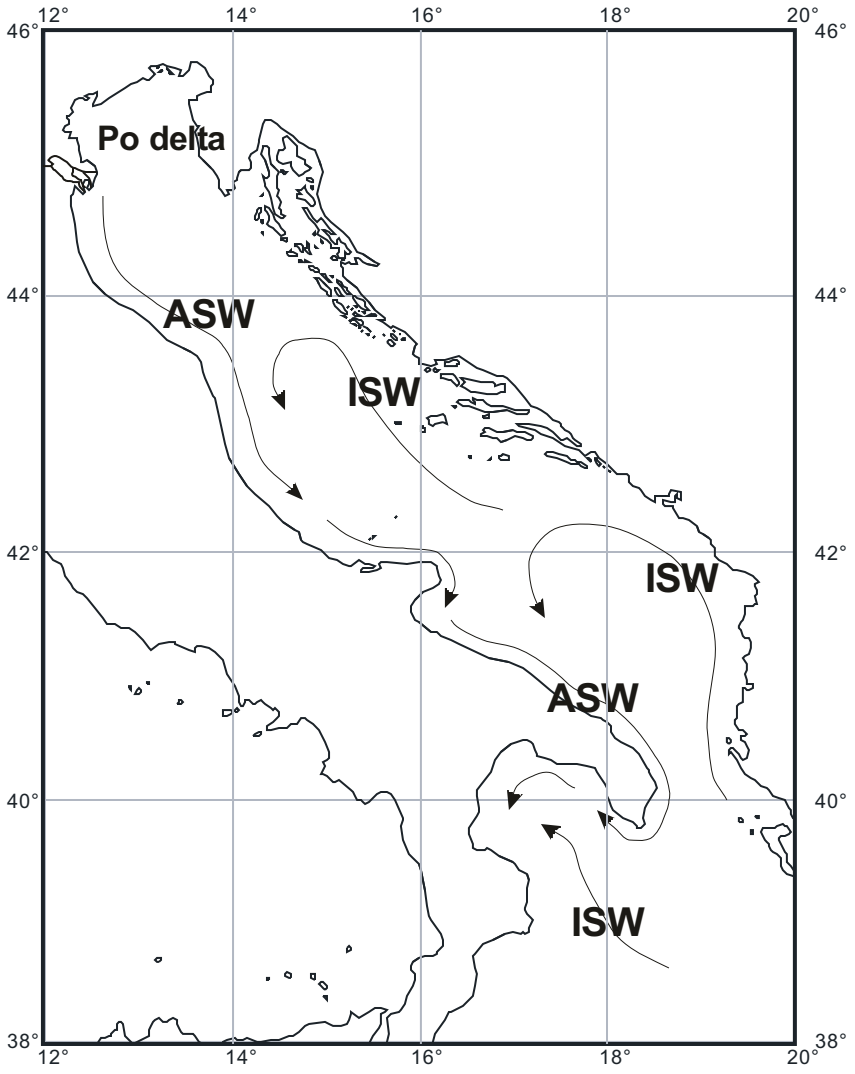

Fig. 1. Map of the upper ocean current system in the research area. ASW=Adriatic Sea surface Water, ISW=Ionian Sea surface Water.

As a result of an anti-clockwise surface water circulation induced by Coriolis forcing, the fresh, nutrient rich Po-river discharge water is pressed against the western coastal margin of the Adriatic Sea (e.g. Lee et al., 2007). As a consequence, a band of enhanced productivity reflected by high chlorophyll-*a* concentration in surface waters, can be observed along the whole western margin of the Adriatic Sea, the Strait of Otranto, around the Calabrian Peninsula into the Golfo di Taranto (Fig. 2). Classically these relative cool, nutrient and suspended matter rich, low salinity waters are classified as Adriatic Surface Water (ASW). Within the Golfo di Taranto, the circulation is cyclonic with both ASW and oligotrophic, high salinity Ionian Surface water (ISW) entering along the eastern part the basin (Fig. 1). Levantine Intermediate Water (LIW) originating from the Levantine basin (Greece) forms the

sub-surface water mass in the Golfo and resides between 150 and 600 m water depth. A shift towards the relatively cool waters of the dense Eastern Mediterranean Deep Water (EMDW) can be observed below about 600m depth (Hainbucher et al., 2006).

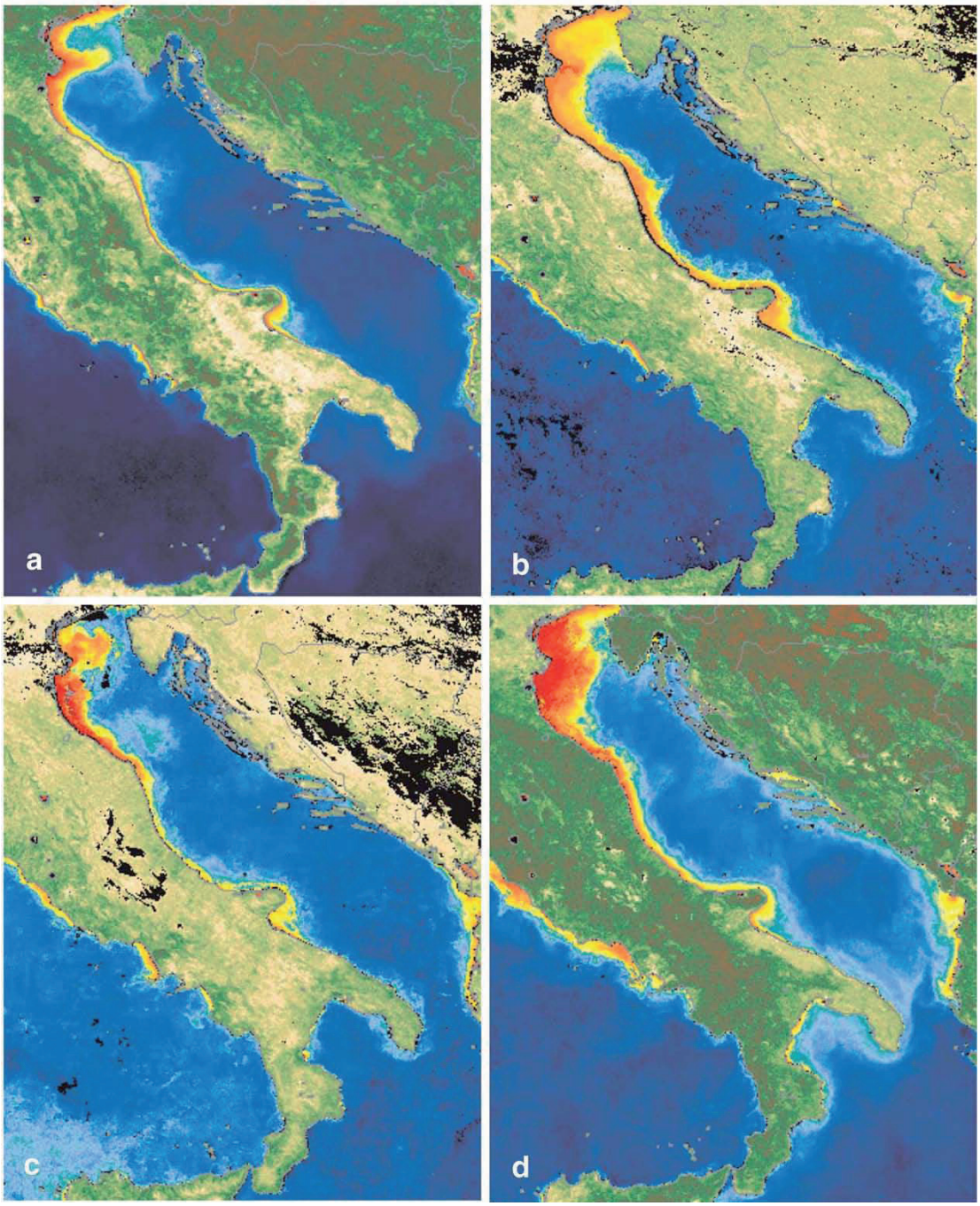

Fig. 2. Compiled satellite image of upper ocean chlorophyll-*a* concentrations in (a) summer, (b) autumn, (c) winter, (d) spring.

Within the Golfo di Taranto both water masses mix with ASW being characteristically maximal traceable as far north as the city of Taranto (Boldrin et al., 2005; Caroppo et al., 2006; Lee et al., 2007; Socal et al., 1999). The surface circulation is strongly dependent on the seasonal cycles, with ISW invading the basin in late winter and early spring. In late spring, summer and autumn, ASW enters the basin from the south-east along the Callabrian margin. During winter enhanced mixing between the oligotrophic ISW and more nutrient enriched LIW resulting in locally enhanced phytoplankton production in upper waters.

4.3 Material and methods

4.3.1 Core Material

Multicore GeoB 10706-3 (lat. 39° 49.50`N, long. 17° 50.00`E) has been collected during the research cruise CAPPUCCINO with the R.V. POSEIDON in June 2006 at 218.3 m water depth (Zonneveld et al., 2008a; Fig. 3). Immediately after recovering, the core has been frozen and stored at -20 °C until further treatment. A total of 46 samples were studied from the 52.6 cm long sediment core. Thirty samples were collected from the upper 96 mm of the core at 3 mm intervals. 15 Samples that origin from the interval between 96 mm – 240 mm, were studied with lower resolution (~ every 9 mm). One “reference” sample has been collected from the base of the core (52.3 cm – 52.6 cm). Previous to sampling the still frozen core was stored at -4 °C for one day enabling high precision cutting that was carried out in a cold storage room at 4 °C. Care was taken that the core temperature remains under -2 °C to avoid damaging of the core structure.

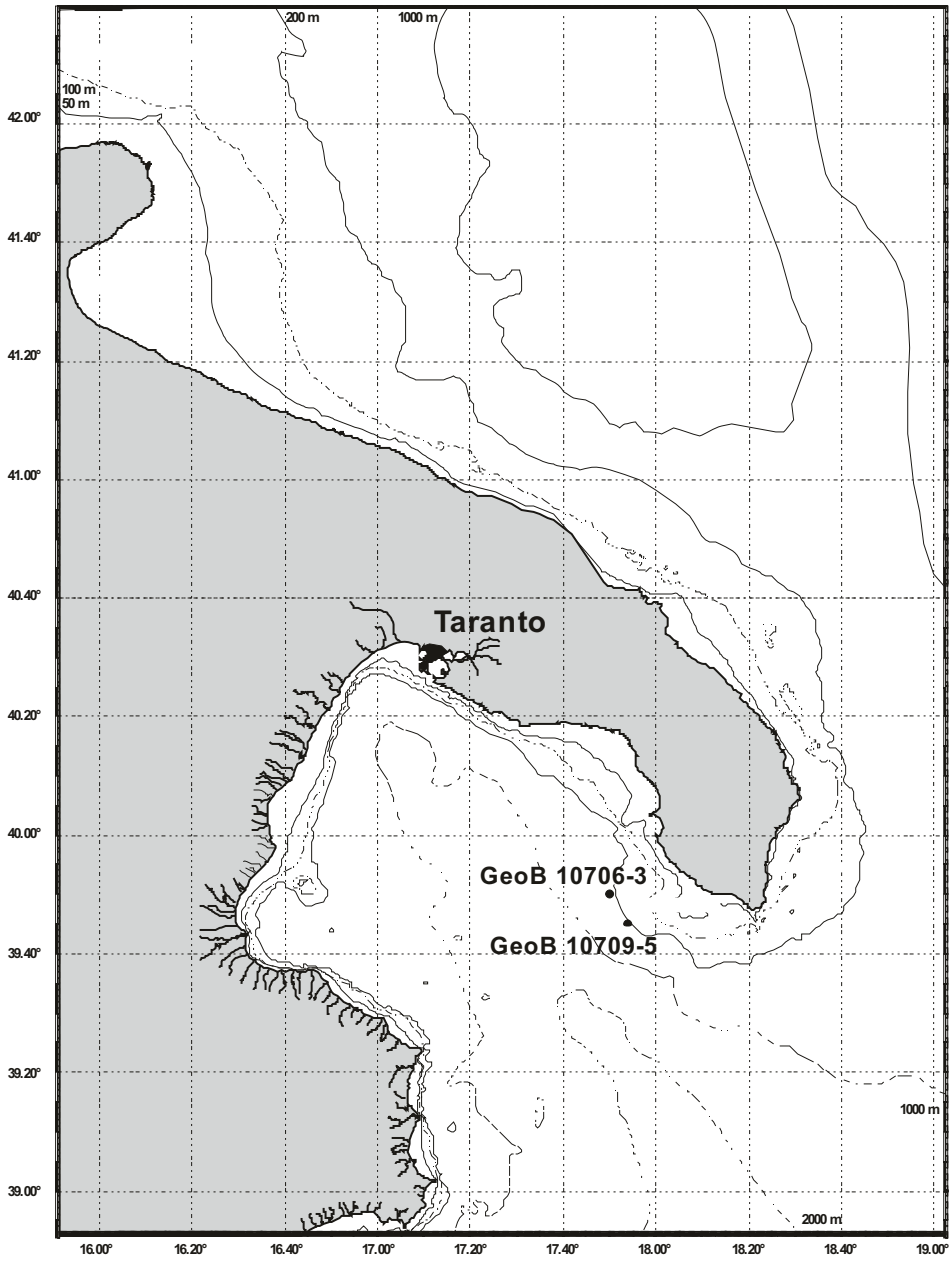

Fig. 3. Locations of the studied core GeoB 10706-3 and core GeoB 10709-5.

4.3.2 Core chronology

The core chronology is based on the age model of the ^{210}Pb-dated multicore core Geo 10709-5 (lat. 39° 45.39`N, long. 17° 53.57`E, 172.3 m water depth) which is located close to the coring site (Fig. 3). Nine slices of sediment core GeoB 10709-5 from depth 0.5, 5, 8, 12, 16, 20, 24, 32, and 34.75 cm were analyzed by gamma spectroscopy at the Institute of Environmental Physics, University of Bremen. Wet samples were sealed into plastic cylindrical dishes with a diameter of 7 cm using Rn

tight foil. Before measurement they were left sealed for a minimum of 3 weeks so that the radioactive equilibrium between ^{226}Ra and ^{222}Rn (and its daughters) has been established. A coaxial HPGe detector Canberra Industries (50% rel. efficiency) housed in a 10 cm Pb shielding with Cu and plastic lining operated under Genie 2000 software was used for gamma spectroscopy. Measurement live-times were 2 days or more. The photopeak efficiencies have been calculated using LabSOCS© (Laboratory SOurceless Calibration System), Genie 2000 software calibration tool, based on defined sample to detector geometry and density. Specific activities of all estimated radioisotopes were recalculated to the date of sampling (18.6.2006).

For determination of excess-^{210}Pb activity ($^{210}Pb_{xs}$) ^{210}Pb-supported activity was subtracted from the ^{210}Pb-total signal, measured via 46.5 keV line. Supported ^{210}Pb was determined via the 351.9 keV line of ^{214}Pb. Additionally, the artificial isotope ^{137}Cs and the natural isotope ^{40}K were analyzed. ^{137}Cs Values above the limit of detection were found in the uppermost 4 slices (down to depth of 12 cm), another value above the limit of detection with high experimental error was observed in the depth of 32 cm. This sample is considered to be an outlier.

The activity is relatively constant in the whole profile, average 12.3 Bq/kg. $^{210}Pb_{xs}$ decreases monotonously in the profile with maximum value of 106 Bq/kg in the depth of 0.5 cm. An exception is an outlier in 32 cm depth (see above). Depth profiles of estimated radionuclides are displayed in Figure 4. An exponential fit was performed on $^{210}Pb_{xs}$ data excluding the uppermost point (obvious signs of bioturbation and oxygen penetration to the depth of 4 cm were recorded during the sampling expedition).

It results in a sedimentation rate of 0.172(38) cm/yr for core GeoB 10709-5. The onset of the nuclear fallout isotope ^{137}Cs in the profile would be expected in the depth of 9 cm (around year 1955), according to this model. However, penetration of the isotope into deeper layers due to continuous mixing at the top of the profile can cause appearance of ^{137}Cs down to greater depth. The age model derived from ^{210}Pb is therefore not in contradiction with ^{137}Cs data.

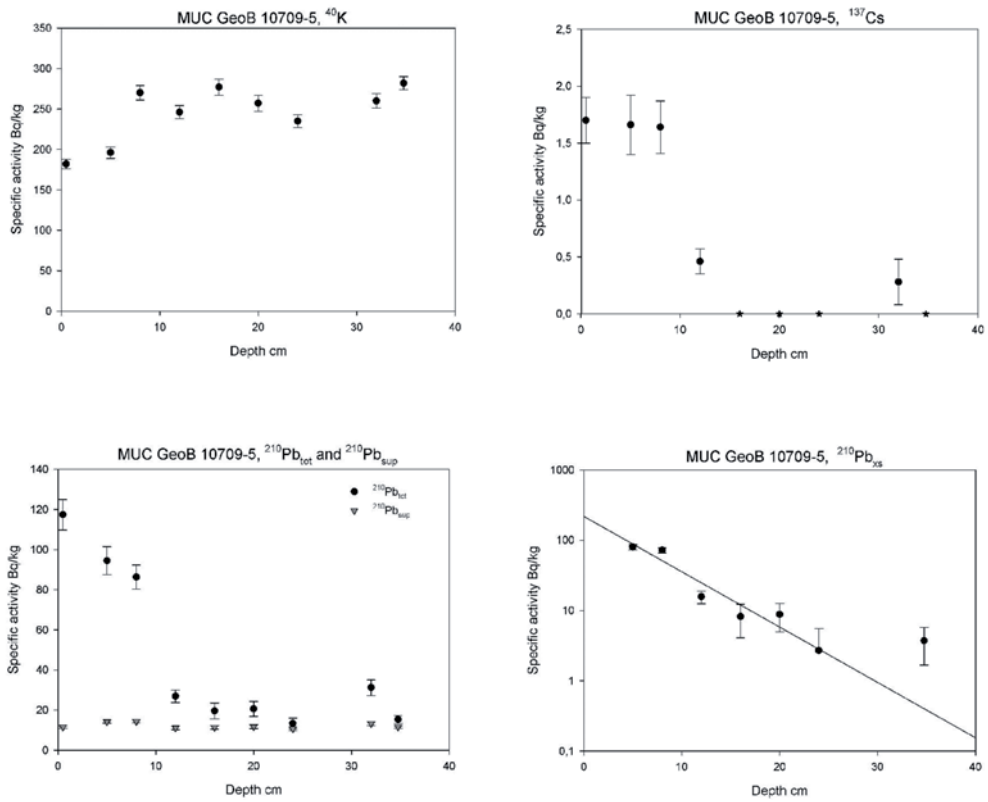

Fig. 4. Depth profiles of (a) ^{40}K and (b) ^{137}Cs (star signs depict measurements with values below decision threshold) (c) supported and total ^{210}Pb and (d) $^{210}Pb_{xs}$ (log-scale). In profiles (a), (b) and (c) also outlier in 32 cm depth is displayed. The 32 cm value is not used for exponential fit in (d). Vertical error bars in all figures stand for 1 standard deviation of the activity including counting statistics and detector calibration uncertainty.

Both cores show a similar succession of dinoflagellate cyst species through time (e.g. *Lingulodinium machaerophorum;* Fig. 5). Since both core sites are in close vicinity of each other and the upper waters consist of the same water mass, these association changes are assumed to have occurred synchronously. The tuning of the dinoflagellate cyst records indicates that a sedimentation rate of 0.3 cm/yr occurs at the core position. Sediments at the base of the core are calculated to represent pre-industrial sediments deposited from year ~1852.

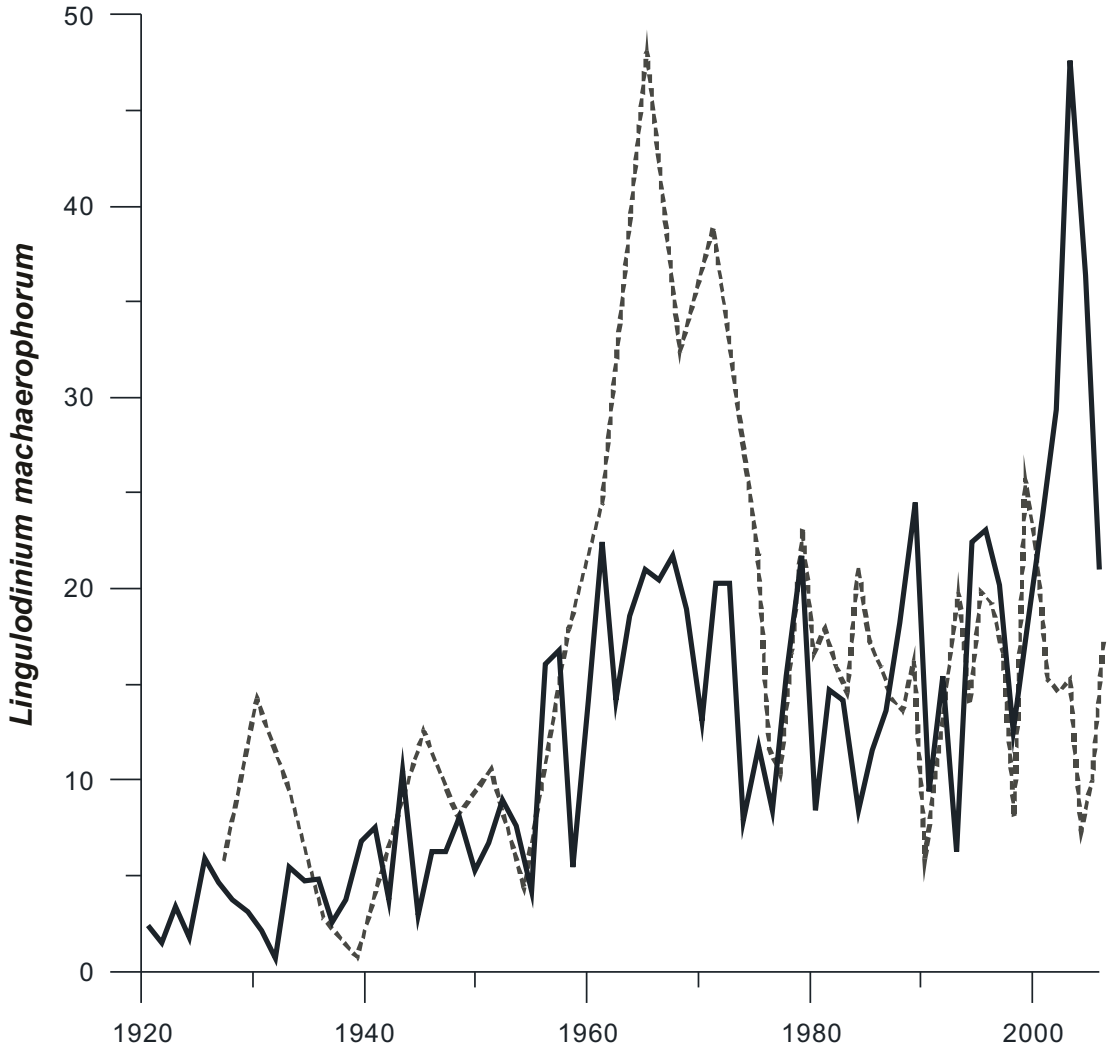

Fig. 5. The synchronous occurrence of *Lingulodinium machaerophorum* in core GeoB 10709-5 (black line) and core GeoB 10706-3 (dotted line).

4.3.3 Palynological processing and geochemical sediment analysis

Palynological preparation has been carried out according to the method described by Elshanawany et al. (2010). For dinocyst analysis, sediment samples were dried at 60 °C, weighed and treated with 10% HCl and 40% HF in subsequent steps; decantation was carried out after each step. Samples were sieved over a 20 µm sieve and the residue was centrifuged (8 min; 3000 rpm) and concentrated to 0.5 ml with a micropipette, subsamples of a known volume (50-100 µl) of homogenized residue were placed on a microscope slide, embedded in glycerine jelly and sealed with paraffin wax. Whole slides were counted for dinocysts using a light microscope with 400 x magnification. When slides contained less than 150 specimens additional

slides were counted. Dinoflagellate cyst accumulation rates were counted (up to 276 specimens in rich samples or with at least ~ 150 specimens in poor samples; Table 1). Taxonomical concept is consistent to that cited in Radi and de Vernal (2008) and Rochon et al. (2009). When possible cyst names are used rather than motile names. The taxonomy of *Polykrikos schwarzii* and *Polykrikos kofoidii* is based on Matsuoka et al. (2009).

0.5 g of the samples was dried, grounded to a fine powder and used to determine total organic carbon (TOC) in the sediment by using carbon analyzer (C – 200, version 2.6).

Table 1. List of all the identified Dinoflagellate cyst species in core GeoB 10706-3. Concentration of dinoflagellate cysts (cysts per gram of dry sediments), Cyst influx (cysts cm^{-2} ky^{-1}), total numbers counted from the core GeoB 10706-3, DBD = dry bulk density (g/cm^3).

(A) Dinocyst in the assemblages	(B) Dinocsyt data				
	Depth (mm)	Cyst influx	Count	Cysts/g	DBD
Ataxodinium choane	0-3 mm	101.3	193	804.7	0.60
Bitectatodinium spongium	3-6 mm	52.5	150	348.9	0.72
Bitectatodinium tepikiense	6-9 mm	96.6	184	642.5	0.72
Brigantedinium spp.	9-12 mm	77.2	147	469.5	0.78
Echinidinium spp.	12-15 mm	113.9	217	670.2	0.81
Gymnodinium catenatum	15-18 mm	118.7	226	677.6	0.83
Impagidinium aculeatum	18-21 mm	93.5	178	575.7	0.77
I. paradoxum	21-24 mm	228.9	218	1,421.5	0.77
I. patulum	24-27 mm	38.6	147	328.2	0.56
I. plicatum	27-30 mm	80.3	153	543.5	0.70
I. sphaericum	30-33 mm	67.2	192	515.5	0.62
I. strialatum	33-36 mm	83.0	158	592.2	0.67
Islandinium (minutum?)	36-39 mm	71.9	137	681.7	0.50
Leipokatium invisitatum	39-42 mm	104.0	198	1,104.2	0.45
Lejeunecysta oliva	48-51 mm	21.4	163	183.3	0.56
L. sabrina	51-54 mm	52.0	198	574.6	0.43
Lingulodinium machaerophorum	54-57 mm	57.5	219	625.0	0.44
Nematospaeropsis labyrinthus	57-60 mm	58.0	221	674.4	0.41

Operculodinium centrocarpum	60-63 mm	74.6	142	787.1	0.45
Operculodinium israelianum	63-66 mm	87.7	167	987.1	0.42
Pentapharsodinium daleii	66-69 mm	119.2	227	1,123.4	0.51
Polykrikos schwarzyii	69-72 mm	89.3	170	1,187.5	0.36
Protoperidinium americanum	72-75 mm	84.0	160	656.1	0.61
Pyxidinopsis reticulata	75-78 mm	114.5	218	946.5	0.58
Quincuesuspis concreta	78-81 mm	94.0	179	836.0	0.54
Selenopemphix nephroides	81-84 mm	102.9	196	911.7	0.54
Selenopemphix quanta	84-87 mm	58.8	224	481.5	0.58
Spiniferites bentorii	87-90 mm	43.1	164	355.0	0.58
Spiniferites mirabilis	90-93 mm	71.9	274	547.8	0.63
Spiniferites ramosus	93-96 mm	72.5	276	621.2	0.56
Spiniferites spp.	105-108 mm	191.1	182	1,248.6	0.73
Stelladinium stellatum	114-117 mm	236.3	225	1,471.0	0.76
Tectatodinium pellitum	123-126 mm	246.8	235	1,627.9	0.72
Trinovantedinium applanatum	135-138 mm	126.0	240	1,477.8	0.41
Tuberculodinium vancampoae	144-147 mm	158.6	151	2,226.5	0.34
Votadinium calvum	156-159 mm	53.9	165	382.8	0.72
Xandarodinium xanthum	165-168 mm	118.6	242	771.4	0.78
	174-177 mm	75.5	154	471.0	0.82
	183-186 mm	126.7	194	902.0	0.72
	192-195 mm	98.0	150	690.1	0.72
	201-204 mm	49.0	150	287.3	0.87
	210-213 mm	75.8	232	499.6	0.77
	219-222 mm	129.4	264	820.2	0.80
	228-231 mm	183.3	187	1,236.3	0.76
	237-240 mm	76.4	156	537.6	0.73
	459-462 mm	173.3	165	947.3	0.87

4.3.4 River outflow and environmental parameters

Information about Po-river discharge is obtained from daily measurements from 1921 – 2000 at a station near Pontelagoscuro, which is situated just upstream the Po-river delta (Tomasino et al., 2004). Information about fertilizer use of Italy between 1961 and 2002 has been achieved from the online dataset “Nation Master” of

agriculture statistics (http://www.nationmaster.com/graph/agr_fer_con_met_ton-agriculture-fertilizer-consumption-metric-tons). Temperatures have been obtained from local Italian climate stations between 1879 and 2000 from http://climexp.knmi.nl/start.cgi?someone@somewhere.

4.3.5 Paleoenvironmental indicators

4.3.5.1 Preservation signal

Information about potential post-depositional aerobic degradation of palynomorphs is estimated using the degradation index "kt" (Versteegh and Zonneveld, 2002) according to the formula:

$kt = \ln (X_i/X_f)$, with $X_i = 68 \times X_f$

(X_f = final cyst concentration of sensitive cysts (cysts/cm^2/ky) and X_i = initial cyst concentration of sensitive cyst species (cysts/cm^2/ky). Resistant and sensitive species are determined according to the division given in Zonneveld et al. (2008b). In this study the group of resistant cysts is formed by the species: *Ataxodinium choanum, Bitectatodinium tepekiense, Impagidinium aculeatum, Impagidinium paradoxum, Impagidinium patulum, Impagidinium plicatum, Impagidinium sphaericum, Impagidinium* spp., *Impagidinium strialatum, Nematosphaeropsis labyrinthus, Operculodinium israelianum,* and cysts of *Pentapharsodinium daleii.* The group of sensitive species consists of *Brigantedinium* spp., *Echinidinium* spp., *Gymnodinium catenatum, Islandinium minutum, Leipokatium invisitatum Lejeunecysta oliva, Lejeunecysta Sabrina, Polykrikos schwarzyii,* cyst of *Protoperidinium americanum*, *Quincuesuspis concreta*, *Selenopemphix nephroides, Stelladinium stellatum, Votadinium calvum,* and *Xandarodinium xanthum.*

4.3.5.2 Trophic state of the upper waters

Information about the trophic state of the upper waters is obtained by determining the total amount of organic carbon (TOC) and calculating total dinoflagellate cyst accumulation rates according to the formula:

AR (cysts/cm^2/ky) = SR (cm/ky) x DBD (g/cm^3) x cysts/g

with SR = sedimentation rate, DBD = dry bulk density.

Detailed studies of the geographical distribution of cyst species in modern sediments of the Adriatic Sea and Golfo di Taranto revealed that the species *Echinidinium* spp., *Lejenecysta sabrina, Lingulodinium machaerophorum, Polykrikos schwarzyii,* and *Stelladinium stellatum* are specifically found in modern day sediments of the Po-river discharge plume (Nichetto et al., 1995; Rubino et al., 1997; Rubino et al., 2000; Sangiorgi et al., 2001; Sangiorgi et al., 2005; Zonneveld et al., 2009). Highest concentrations of these species are nowadays found close to the river mouth. Accumulation rates of these species are used to obtain information about plume water characteristics.

4.3.5.3 Past sea surface temperature (SST)

Qualitative information about variations in sea surface temperature is obtained using the following ratio:

$$W/C = W_n / (W_n + C_n)$$

where n = number of specimens counted, W = warm water species, C = cold water species.

W = *Impagidinium aculeatum, Impagidinium paradoxum, Impagidinium patulum*, *Impagidinium strialatum, Operculodinium israelianum,* and *Spiniferites mirabilis.* C = *Bitectatodinium tepikiense.* Species selection is based on the global geographic distribution (e.g. Marret and Zonneveld 2003).

4.4 Results

Accumulation rates of sensitive and resistant cyst species vary synchronously throughout the core resulting in a relatively constant kt of values that remain below 4.4. (Fig. 6).

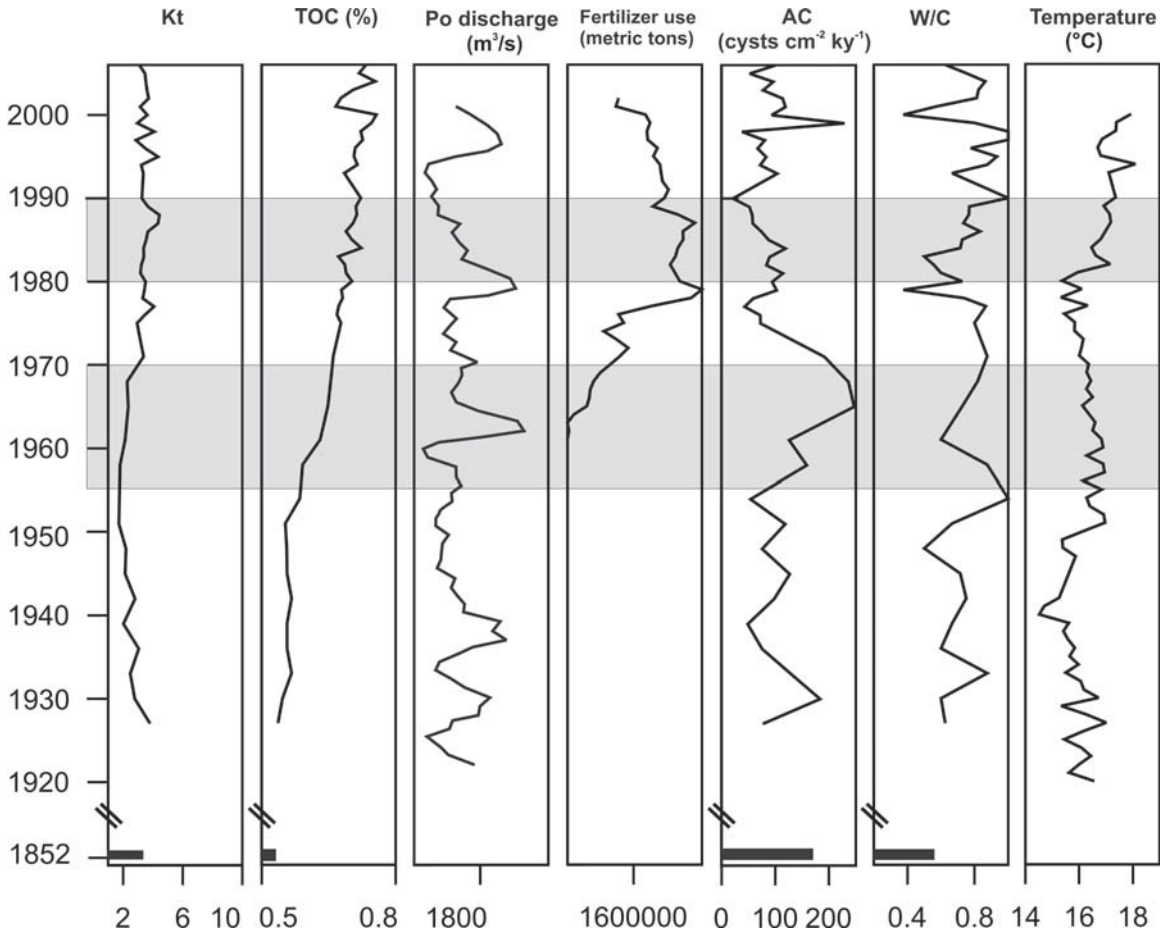

Fig. 6. Downcore variations in GeoB 10706-3 of kt, percentages of total organic carbon (TOC %), Po river discharge (m^3/s), fertilizer use (metric tons), total dinoflagellate accumulation rate (AC; cysts cm^{-2} ky^{-1}), W/C dinoflagellate signal, air temperature (°C). Black bars indicate the core base.

Based on variations in the TOC and dinoflagellate cyst association five prominent phases can be distinguished:

Phase 1: ~1852 and 1927 – 1955

The lowest concentration of total organic carbon (TOC) can be observed at the core base (background sample; 0.528%). From 1927 to 1955 TOC concentrations are relatively constant with mean values of 0.555%.

Total cyst accumulation rates are relatively low but show a short maxima at 1930 (Fig. 6). Total accumulation rates fluctuate around intermediate values after this date until 1955.

Between 1927 and 1955 cyst concentrations of *Echinidinium* spp., *Lingulodinium machaerophorum,* and *Stelladinium stellatum* are relatively low (Fig. 7). *Lejeunecysta sabrina* and *Polykrikos schwarzyii* are absent from the association (Fig. 8). The dinoflagellate cyst association is dominated by phototrophic species, notably by *Operculodinium centrocarpum* (Fig. 9).

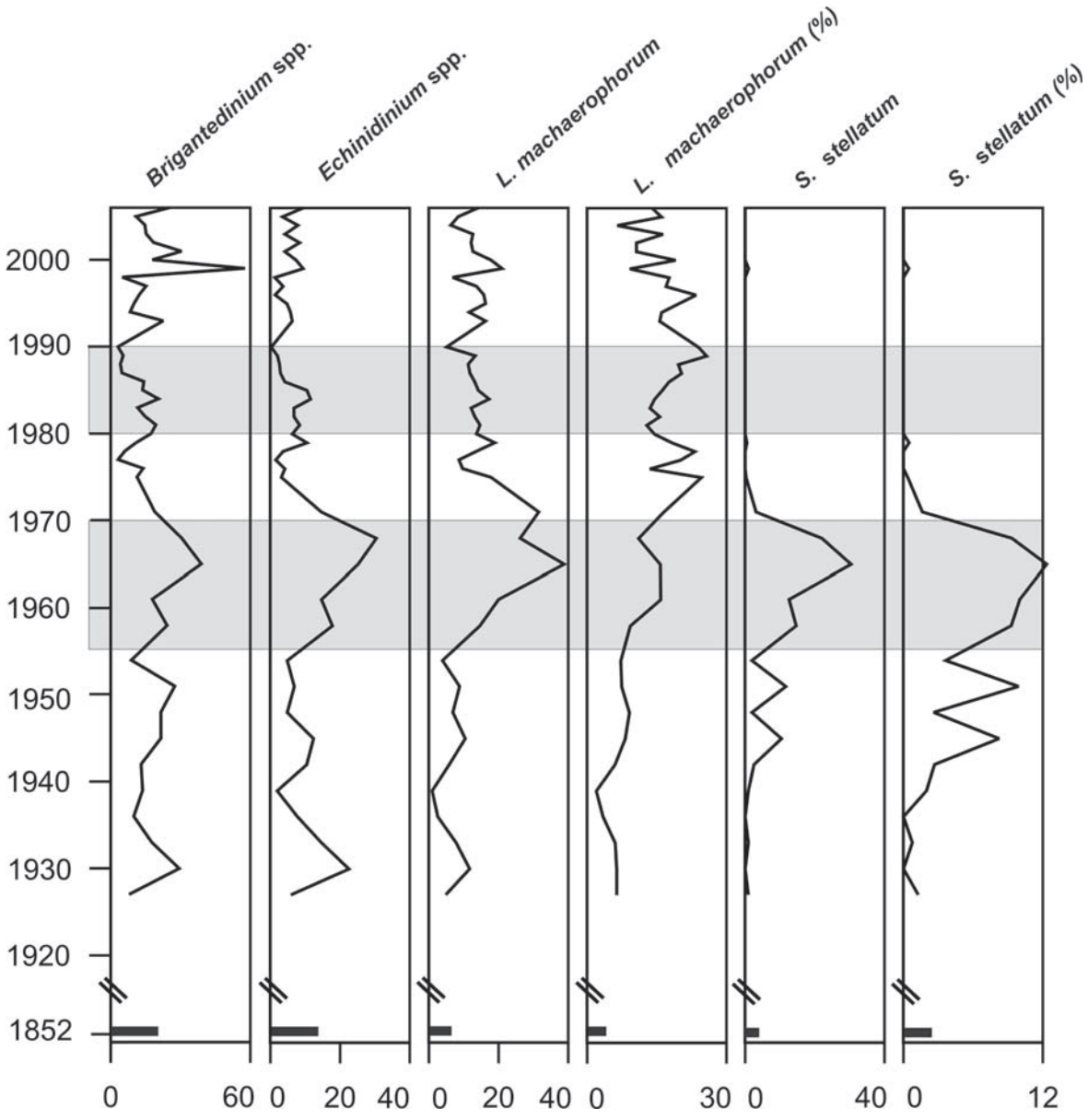

Fig. 7. Accumulation rates (cysts cm^{-2} ky^{-1}) and relative abundances (%) of organic-walled dinoflagellate cyst taxa from core GeoB 10706-3. Black bars indicate the core base.

Relative abundances of *O. centrocarpum* show a decreasing trend from 1930 onward accompanied by an increasing trend on relative abundances of *L. machaerophorum* and *S. stellatum* (Figs. 7, 9).

W/C ratio is relatively low around 1852. It fluctuated around intermediate values with short positive pulses at 1933 and 1942 (Fig. 6).

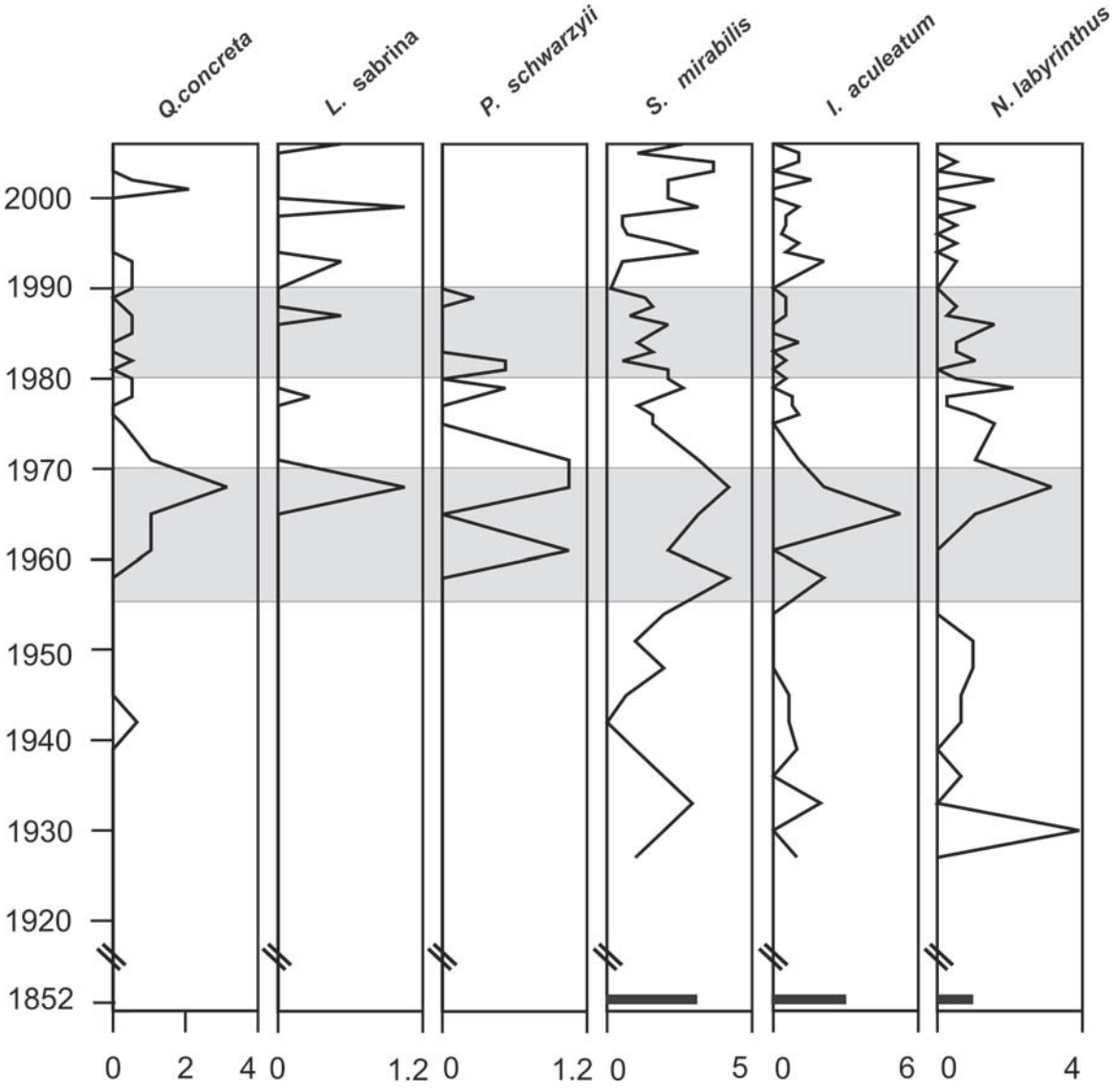

Fig. 8. Accumulation rates of organic-walled dinoflagellate cyst taxa from core GeoB 10706-3 given in cysts cm^{-2} ky^{-1}. Black bars indicate the core base.

Phase 2: 1955 – 1970

This phase is characterized by a strong increase of TOC values, as well as by increasing total cyst accumulation rates. A considerable amount of the observed dinoflagellate cyst species such as *Brigantedinium* spp., *Echinidinium* spp., *Impagidinium aculeatum, Lingulodinium machaerophorum, Nematosphaeropsis labyrinthus, Operculodinium centrocarpum*, cysts of *Pentapharsodinium daleii, Quencususpis concreta, Spiniferites mirabilis,* and *Stelladinium stellatum* follow a similar trend (Figs. 7, 8, 9). This increase is most pronounced for *Echinidinium* spp., *L. machaerophorum,* and *S. stellatum* which reach maximum values in 1968, 1965, and 1965 respectively. Contemporaneous to this increase *Polykrikos schwarzyii* occurs for the first time in the dataset (1961) followed by *Lejeunecysta sabrina* in 1968. Although accumulation rates of *O. centrocarpum* show a similar pattern, its relative abundances decrease steadily in this interval. Relative abundances on *L. machaerophorum* and *S. stellatum* increase markedly in this interval (Fig. 7).

W/C ratio slightly increases to higher mean values showing a maxima at 1955 and minimum at 1961 (Fig. 6).

Phase 3: 1970 – 1979

During this phase TOC values still increase but less pronounced than during the previous phase. Cyst accumulation rates decrease to reach a minimum in 1977 (Fig. 6).

This phase is typically characterized by strongly decreasing accumulation rates of *Stelladinium stellatum* followed by *Echinidinium* spp., *L. machaerophorum, O. centrocarpum,* and *Polykrikos schwarzyii* in the late 70's (Figs. 7, 8, 9). With the exception of three single cyst findings, *S. stellatum* is absent from the association after 1971. *Lejeunecysta sabrina* forms a rare but constant member of the cyst association. Cyst concentrations of cysts of *Pentapharsodinium daleii* remain high throughout this interval. *Lingulodinium machaerophorum* remains however the most dominant species of the association (Fig. 7).

The W/C ratio shows relatively high values to drop abruptly to minimum value in 1979 (Fig. 6).

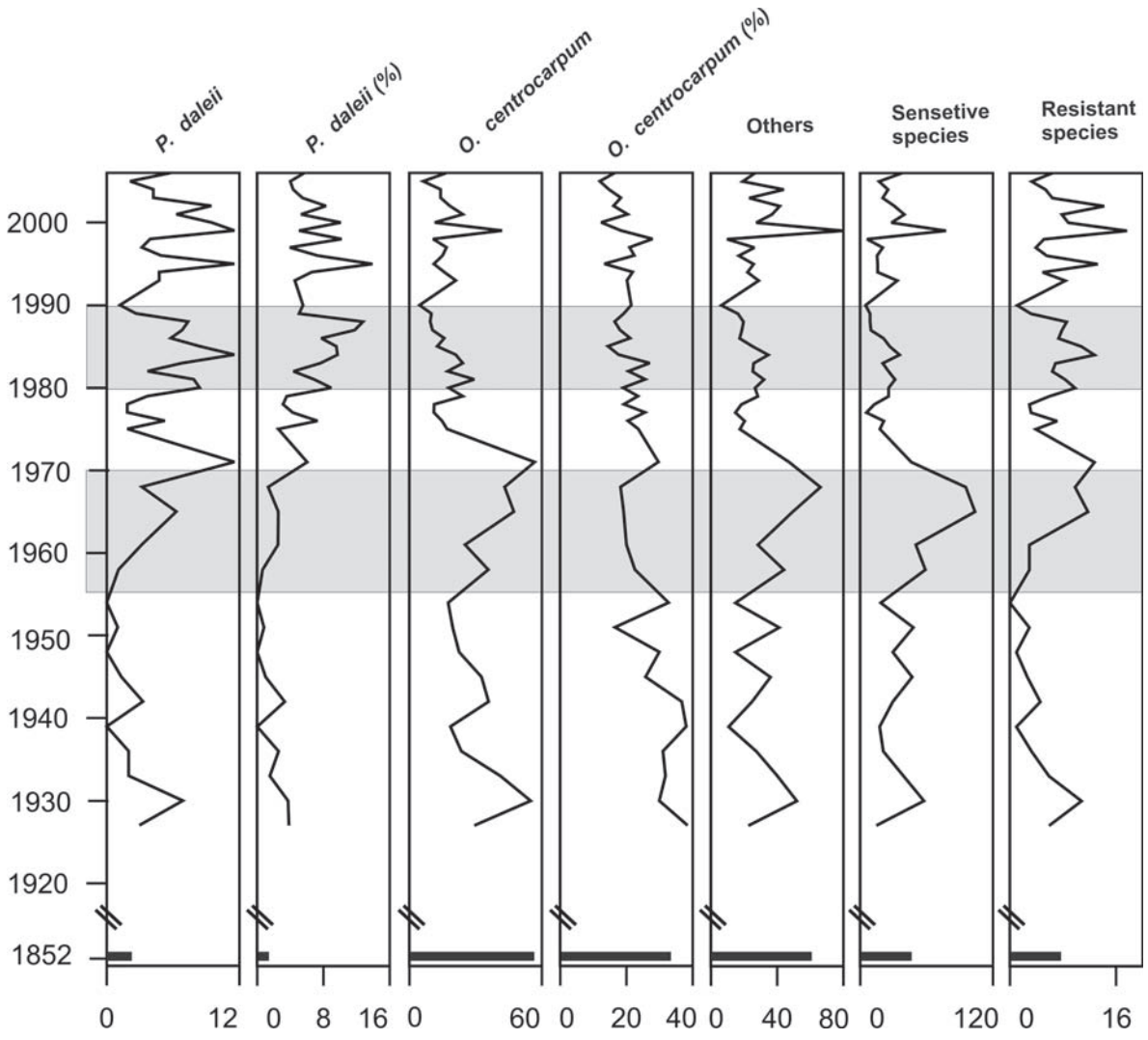

Fig. 9. Accumulation rates (cysts cm^{-2} ky^{-1}) and relative abundances (%) of organic-walled dinoflagellate cyst taxa from core GeoB 10706-3. Black bars indicate the core base.

Phase 4: 1980 – 1990

This phase is characterized by only slowly increasing TOC values. Total accumulation rates show a peek of enhanced values between 1981-1990 (Fig. 6). Accumulation rates of *Echinidinium* spp., *L. machaerophorum,* and *Operculodinium centrocarpum* follow a similar pattern but their values are considerably lower compared to the previous two phases (Figs. 7, 9). Accumulation rates of cyst of *Pentapharsodinium daleii* remain high throughout this interval. Relative abundances of *L. machaerophorum* become stable whereas those of *P. daleii* increase (Figs. 7, 9).

The W/C fluctuated around intermediate values with short maxima at 1986 (Fig. 6).

Phase 5: 1990 – 2006

This phase is characterized by relatively high TOC values that show only a slight increasing trend. Total cyst accumulation shows a short peek between 1995-2004. *Polykrikos schwarzyii* disappears from the association and is not registered in samples deposited after 1990. The interval is characterized by decreasing relative abundances of *L. machaerophorum* (Fig. 7).

The W/C fluctuated around intermediate values with short maxima at 1997/1998 and 2003 and a minimum at 2000 (Fig. 6).

4.5 Discussion

Within the last years it has become evident that early diagenetic aerobic degradation can severely affect the dinoflagellate cyst association post-depositional (Zonneveld et al., 1997, 2001; Persson and Rosenberg, 2003; Zonneveld et al., 2010a. Shipboard observations registered a visible oxygen penetration depth of only 1 to 2.5 cm (Zonneveld et al., 2008a). This indicates that cysts in bottom sediments could have been exposed to oxygen for about 3 to 8 years. Recent studies on degradation rates of dinoflagellate cysts in natural environments have documented a selective loss of about 30% of sensitive cyst species after 15 months of exposure to oxygenated conditions whereas resistant cyst species were not affected (Kodrans-Nsiah et al., 2008). If species selective degradation had affected our association post depositional, we would expect to observe a rapid decrease in cyst concentration of sensitive cysts in the upper few centimetres whereas resistant cysts species do not show a change. We do not see such a difference (Fig. 6). On the contrary the kt-index remains rather constant throughout the whole core. We therefore expect that preservation of cysts is excellent and that changes in the dinoflagellate cyst accumulation rates and association reflect changes in the upper water column.

Studies in natural environments reveal that maximal cyst production occurs during or just after dinoflagellate cyst blooms (Wall and dale, 1968; Ishikawa and Taniguchi, 1996; Montresor et al., 1998; Kremp and Heiskanen, 1999; Godhe et al., 2001).

Recent studies have shown that in oligotrophic/mesotrophic environments cyst production of generally all dinoflagellate species is positive related to increasing nutrient availability in surface waters (see overviews in e.g. Zonneveld et al., 2010b; Shin et al., 2010; Elshanawany et al., 2010). That this hold as well for our research area is indicated by study on two inner-bay sites in the "Mar Grande" and "Mar Piccolo" located near the city of Taranto at the proximity of the area where river plume waters can be traced (Rubino et al., 1998). Within these bays maximal cyst production is observed when enhanced concentrations of dinoflagellates occur in the upper waters related to enhanced upper water nutrient concentrations. Since we have no indication of derivative overprint, we can assume that our dinoflagellate cyst accumulation rates reflect cyst production and as such upper water dinoflagellate production, which in turn can be related to the trophic state of the upper waters.

Nutrients and trace elements can be brought into the research area by aeolian transport, as a result of winter mixing of surface waters with LIW or by inflow of ASW. In turn aeolian particles can be transported into the region by winds or in the form of volcanic ash. During the time interval of this study, major eruptions of the Mount Edna and Vesuvius occurred in 1928, 1949, 1971, 1983, 1992, 2000 (Mt. Edna) and 1944 (Vesuvius). We observed no changes in TOC, accumulation rates of dinoflagellate or changes in cyst associations at times of these eruptions. Correggiari et al. (2007) reported that the metals deposited from the atmosphere to the Adriatic basin are a small fraction (2-5%) of those discharged by the Po and other rivers. We therefore assume that aeolian transport is not the major source of nutrients in the research area.

In winter, wind induced mixing of surface and intermediate waters enhance nutrient concentrations in the photic zone resulting in enhanced phytoplankton production. Due to the geographic configuration of the basin this occurs in the north and north-western part of the basin. Phytoplankton studies show that these winter blooms are formed notably by coccolithophorids. This is the same at our sampling location. Here dinoflagellates form a considerable part of the plankton during the whole year but with minimum concentrations in November to March and highest concentrations in spring and autumn (e.g. Caroppo et al., 1999; Caroppo et al., 2006). Consequently winter mixing does not seem to influence the dinoflagelate production in the region.

The above mentioned plankton surveys reveal that changes in dinoflagellate cyst production in the region is strongly related to variability in inflow of plume waters (ASW). ASW contains relatively high nutrient/trace-element concentrations compared to the oligotrophic Ionian Sea surface Waters. We therefore can expect that changes in total accumulation rates reflect changes in ASW influx, which is strongly related to river discharge. The Po-river is the main water source of the ASW. On its track southwards it is however spiced downstream with waters from numerous local eastern Italian rivers as well. Although it is quite unlikely that Po-discharge waters reach the sample position, increase discharge of this river would result in an extension of the river plume and a northward shift of ASW waters in the Golfo di Taranto. We indeed find a strong correlation between total cyst accumulation rates and Po-river flux-rates as measured at Pontelagoscuro. We therefore assume that the variability in cyst accumulation rates is mainly influenced by changes in the flow of ASW (Fig. 6).

In several studies the possible role of changes in upper water temperature influencing the phytoplankton production in upper waters of the research area have been discussed (e.g. Caroppo et al., 1999; 2006). Unfortunately no detailed information about surface water temperatures is available for the region. Recently Versteegh et al. (2007) showed that there is a strong relationship between SST and region air temperature. We observe a strong correlation between our ratio of warm and cold dinoflagellate cysts and regional air temperatures as well suggesting that our W/C ration can be used to reconstruct past SST. We observe no correlation between changes in cyst accumulation rates or major changes in the cyst association composition with variability in the W/C ratio. We therefore assume that the observed changes in cyst accumulation rates of individual species are not the result from temperature changes in the upper waters.

On top of our signal reflecting fluctuations in river outflow we observe a change in association composition. From 1932 onward the species *Lingulodinium machaerophorum* becomes an increasing prominent part of the association. *L. machaerophorum* is typically found in environments of high nutrient concentrations and is found to be a good indicator for (anthropogenic induced) eutrophication in estuarine environments (e.g. Sangiorgi et al., 2004; Dale, 2009; Shin et al., 2010).

This suggests that water quality is already changing towards more eutrophic conditions from 1932 onward. This is confirmed by the only long-term dinoflagellate cyst study of the region that is currently present (Sangiorgi and Donders, 2004). Sangiorgi and Donders (2004) observe an increase in relative abundance values of *L. machaerophorum* since 1930 consistent with our findings.

From 1955 – 1970 we observe a remarkable increase in the cyst production of a number of species that is much higher as can be expected as a result of increased ASW inflow of the basin only (Fig. 6). This change in association is accompanied by a contemporaneous abrupt increase in TOC concentrations. Previous to this date the dinoflagellate cyst association is dominated by *Operculodinium centrocarpum* whereas after this date *Echinidinium* spp. *Lingulodinium machaerophorum,* and *Stelladinium stellatum* form a major part of the association. Contemporaneous to this change *Polykrikos schwarzyii* occurs for the first time in the dataset shortly followed by *Lejeunecysta sabrina*. Studies on the geographic cyst distribution in modern sediment reveal that *O. centrocarpum* is a cosmopolitan species thriving in oligotrophic to mesothrophic environments. It has been suggested to be especially present in areas characterized by unstable surface water conditions (e.g. Marret and Zonneveld, 2003; Verleye et al., 2009). *Echidinium* spp., *L. machaerophorum* and *S. stellatum* are typically found in high abundances in eutrophic environments (e.g. Marret and Zonneveld, 2003). Together with *Polykrikos schwarzyii* an increase in their concentrations can often be related to anthropogenic-induced eutrophication, mainly as result of enhanced input of notably nitrate and phosphates to the environment (e.g. Pospelova et al., 2002, 2005, Matsuoka et al., 2003; 2005; Dale, 2009; Shin et al., 2010). In the Adriatic Sea and Golfo di Taranto *Echinidinium* spp., *L. macharophorum*, *S. stellatum*, *P. schwarzyii* and *S. stellatum* are typically found in the ASW with highest concentrations close to the Po-river mouth where highest nutrient concentrations of e.g. nitrate, ammonia and phosphate are found (Sangiorgi et al., 2002; 2005; Zonneveld et al., 2009). Our results therefore suggest a strong increase in nutrient concentrations of the ASW after 1955. This confirms the present limited amount of information that contains digital data about physical conditions in the region from time intervals previous to 1970. The longest eutrophication records that are currently available focus on the oxygen conditions and dinoflagellate cyst associations in the Northern Adriatic Sea (Justic, 1987; Sangiorgi and Donders

2004). First indications for oxygen depletion near the sea bottom are reflected by data gathered between 1955-1966 (Justic, 1987). Justic (1987) concludes this to be strongly related to phosphate concentration increase in surface waters. Sangiorgi and Donders (2004) reconstruct maximal eutrophication to occur around 1966. Digital data about the amount of fertilizer use in Italy show a strong increase in the 1960's (Fig. 6). The Po-plain and areas at the east side of the Appenines form by far the major part of the Italian agriculture land-use and as such the trends in total fertilizer use reflects the use in the drainage areas of rivers spicing the ASW. In Italy the use of fertilizer forms the major source for nitrate and phosphate concentrations in the discharge waters (e.g. de wit and Bendoricco, 2001). We therefore suggest that the strong change in TOC and dinoflagellate cyst association seen in the early 1960's can be subscribed to a change in quality of the river water on top of a change in quantity.

Between 1970 and 1979 we document a decrease in accumulation rates of the species that are characteristic for ASW waters contemporaneous to a decrease in Po-outflow waters. With exception of changes in absolute and relative abundances of *S. stellatum* we see however no strong changes in association composition. We therefore assume that the changes in accumulation rates are the result of the quantity of ASW inflow in the research area. The slight change in association composition might be related to the quality of the composition of the ASW waters though. For this time interval a doubling of the nutrient load carried by the Po-river has been estimated and episodes of severe anoxia have been recorded in north Adriatic Sea (e.g. Justic et al., 1987; Marchetti et al., 1989; Degobbis, 1989; Degobbis and Gilmartin, 1990). Based on these observations we suggest that this extreme eutrophication of ASW waters might have been unfavourable for *S. stellatum.* To date there is unfortunately not much known about the ecology of *S. stellatum* and more studies are needed to test this hypothesis (Marret and Zonneveld., 2003; Shin et al., 2010). This interval is characterized by highest relative abundance values of *L. machaerophorum*. In the North Adriatic Sea

From 1980 onward we see stabilizing TOC concentrations and cyst accumulation values, with the trend in cyst accumulation rates following Po-river discharge rates. Relative abundances of *L. macharophorum* remain stable and decrease after 1990. In 1990, *P. schwarzyii* even disappears from the association. This suggests that

although total cyst accumulation rates still reflect river ASW inflow rates the association change reflects a stabilization of nutrient concentration condition of surface waters between 1980 and 1990 and an improvement of the water quality after 1990. This is in agreement with observation in the North Adriatic Sea where for this time interval a decreasing trend in upper waters chlorophyll-*a* concentrations and phytoplankton production is registered. (Harding et al., 1999; Rinaldi et al., 1998; Mozetiç et al., 2010). This reduction is subscribed to the decreasing concentrations in phosphorus concentrations of the Po-river outflow waters that are banned by Italian law since the mid 1980s (de Wit and Bendrocchio 2001; Degobbis et al., 2000; Solidoro et al., 2009).

In contrast to *L. machaerophorum* and *P. schwarzyii,* the species *Pentapharsodinium daleii* does not show a reduction in cyst production. For the time interval a strong reduction in both ammonia and phosphate but not in nitrate has been documented (see overview in Solidoro et al., 2009). We therefore assume that *P. daleii* is not that sensitive for changes in phosphate or ammonia concentrations and that the high amounts of nitrate in the water might be favourable for this species for its cyst production. Although our results suggest a recent improvement of the water quality the high TOC concentrations and accumulation rates of cyst species characteristic for eutrophic regions indicates that ASW waters are still eutrohicated. More measurement might therefore be needed to improve the water quality considerably.

4.6 Conclusions

As result of the extreme good preservation of dinoflagellate cyst in sediments of the south-eastern part of the Golfo di Taranto, variability of cyst accumulation rates reflect changes in cyst production in surface waters. Changes in cyst production reflect changes in quantity of plume water inflow (ASW) into the basin. A change to more eutrophic conditions of the regions can be observed already in the lowest part of the core deposited between 1932 and 1955. A strong change towards eutrophic condition is found between 1955 – 1970 caused by a combination of enhanced river discharge in the ASW source areas and anthropogenic activities. From 1980 onward, notably after 1990, water quality improves slightly probably as the result of reduced phosphate concentrations in the upper water masses.

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Chapter 5

Conclusions

The results compiled in this work investigate the modern distribution of dinoflagellates and benthic foraminifera in the Mediterranean Sea, with special focus in the Po-river system, and one of the polluted hot spot areas (Abu-Qir Bay, in the south-eastern Mediterranean Sea). This provide us with the information required to reconstruct eutrophication history and to obtain insight in the influences of human and natural changes on marine ecosystem as a result of ongoing population growth, increased nutrient runoff by rivers, and nutrient quality of the pollution sources.

Our investigations on modern sediments from the Mediterranean Sea indicate that the organic-walled dinoflagellate cysts are sensitive recorders of both upper and bottom Mediterranean water characteristics. Based on the relative abundance data, two groups can be distinguished that are characteristic for the major oceanographic settings in the region; 1) the offshore eastern Mediterranean Sea group where surface sediments are characterised by oligotrophic, warm, high saline surface waters and oxygen rich bottom waters. The cyst association is characterized by the dominance of *Impagidinium aculeatum, Impagidinium sphaericum, Impagidinium patulum, Impagidinium paradoxum, Impagidinium* spp., *Impagidinium plicatum, Pyxidinopsis reticulata, Nematosphaeropsis labyrinthus* and *Operculodinium israelianum*. Temperature forms a causal factor influencing the production of *Operculodinium israelianum*, rather than for the other species in this group. *Impagidinium* species and *Nematosphaeropsis labyrinthus* show a positive relationship between cyst accumulation and nitrate availability in upper waters. 2) the western Mediterranean Sea, Strait of Sicily/NW Ionian Sea and at the distal end of river plumes group, where surface waters are characterized by high primary productivity associated with low SST and SSS, whereas bottom water oxygen concentrations are relatively low. The cyst association consists of *Selenopemphix nephroides, Echinidinium* spp., *Selenopemphix quanta, Quinquecuspis concreta, Brigantedinium* spp. and *Lingulodinium machaerophorum*. Relative abundances and accumulation rates of *Selenopemphix nephroides* have a positive relationship with upper water chlorophyll-

a and nitrate concentrations suggesting that this species could form valuable eutrophication indicator in the study area. *Lingulodinium machaerophorum* is found in high abundances in samples located in river discharge plumes especially in the Nile plume. It can be used as a suitable marker to trace past variations in river discharge, especially from the Nile.

The composition of benthic foraminifera assemblages as well as changes in their concentrations reflect changes in the anthropogenic effects and quantify the degree of pollution in one of the most polluted embayment in the south-eastern Mediterranean Sea. Statistical analyses performed on species with a relative abundance higher than 5% led to the identification of two assemblages. The assemblage that is found in the shallowest most polluted sites is represented by the pollution opportunists species such as*: Ammonia tepida, Quinqueloculina lata,* and *Porosononion* spp. A second assemblage found in off shore less polluted sites is characterised by *Quinqueloculina vulgaris, Elphidium* spp., *Asterigerinata mamilla, Rosalina macropora, Ammonia beccarii, Triloculina trigonula, Peneroplis pertusus,* and *Quinqueloculina* spp. Reduced population diversity, increase in dominance, and the frequent presence of deformed tests were recorded as a result of heavy metal pollution. We show that in the Abu-Qir Bay benthic foraminifera reflect human-induced environmental changes and they can be used as bioindicators for monitoring coastal pollution.

On annual timescales, the 80 years eutrophication history of the distal part of the Po-river discharge plume has been reconstructed through dinocyst analysis together with environmental data and geochemical measurements (TOC) of a marine sediment core GeoB 10706-3. Cyst records have proved useful indicators of human influence. An increase in relative abundances of the species *Lingulodinium machaerophorum* which nowadays is characteristic for nutrient enriched waters in estuarine environments indicates a change in water quality already as early as 1932. From 1955 – 1970 we reconstruct a strong change in water quality towards more eutrophic conditions based on the strong increase in accumulation rates of species that are characteristic for eutrophic environments such as *Echinidinium spp., Lingulodinium machaerophorum,* and *Stelladinium stellatum* and to a lesser extent *Brigantedinium* spp., *Polykrikos schwarzyii, Quincususpis concreta,* and *Lejeunecysta sabrina.* This increase corresponds to enhanced fertilizer use in Italy and first occurrence of

eutrophication related anoxic events in the North Adriatic Sea. From 1980 onward we observe a stabilisation of TOC concentrations, cyst accumulation rates and association composition followed by a decrease in relative abundances of the nutrient indicators *L. machaerophorum* and *P. schwarzyii* suggesting a slight improvement of the water quality. We assume this improvement to be related to a reduction in phosphate concentrations in river discharge waters as a result of governmental measurements.

The manuscript given in the appendix focus on different pelagic eutrophication indicators of the phytoplankton community especially dinoflagellates in Abu-Qir Bay. Water sampling was carried out seasonally throughout the period from May 2005 to February 2006. Nine stations were chosen to cover the main ecological areas and different pollution facets of the bay. Different physical and chemical parameters were measured, including heavy metal concentration, temperature, salinity, nutrient concentration, chlorophyll-*a*, dissolved oxygen, pH, and water transparency, parallel to the phytoplankton analysis. Alternate dominance of the main phytoplankton groups in the population seem to be controlled by changes in the different environmental parameters. During high temperature-salinity and stratified condition dinoflagellates (mainly *Gymnodinium* spp.) become dominant in Abu-Qir Bay during summer. As the temperature dropped to its minimal value in winter, the water column becomes unstable and diatoms become the dominant group of the phytoplankton. The fresh water algae are dominant at times and places of low salinity. Several signs are recorded that can be used as indication of water deterioration in the study area such as: the massive increase of phytoplankton standing crop, reduced diversity, frequent occurrence of toxic dinoflagellate harmful species, and the concurrent pattern between the phytoplankton and zooplankton cycles. Heavy metal pollution has a marked negative effect on dinoflagellate production, which is reflected by low dinoflagellate abundance in the polluted station. The eurythermal euryhaline *Prorocentrum minimum* seems to be adapted to low salinity and temperature, but high nutrient concentration. *Prorocentrum* species, mainly *P. micans* and *P. triestinum*, can be used as indicators for eutrophication, whereas *Ceratium* spp. can be considered as pollution sensitive species.

Chapter 6

Future perspectives

The present study has shed more light into the use of different proxies for reconstructing oceanographic and environmental changes leading to eutrophication in the Mediterranean. This work highlights the large potential applicability of both benthic foraminifera and dinoflagellate cysts in assessing the human and natural influence in aquatic ecosystems. However, further research is needed to test and develop the presented ideas and future improvements may be achieved by the following suggestions.

1- Although a lot of environmental studies have been carried out on dinoflagellates in the last years, the observations are still restricted mainly to their relative abundance rather than absolute abundance. In the present study, accumulation rate data gave us a new picture of dinoflagellate ecology (Chapter 2). Available information of sedimentation rate allowed us to calculate accumulation rate for a limited number of stations (9). Therefore, extending the observations on the accumulation rate will possibly increase the still incomplete basic knowledge about dinoflagellates ecology and thus lead to more reliable reconstructions. The dating of surface sediment samples in order to calculate dinocyst accumulation rates is an important recommendation arises from this study.

2- This study confirms the responses of benthic foraminifera to pollution in Abu-Qir Bay (Chapter 3). Since dinoflagellate cysts distribution has not been investigated yet in this bay and in other highly polluted coastal basins, more surveys are needed in the coastal areas of Egypt. In future, precise coring expeditions will be planned to regions in the vicinity of pollution sources at sites that are characterised by high sedimentation rates to recover core material that allows high temporal resolution studies on the past trophic history of the regions. Literature surveys reveal that these places can be found in the Abu-Qir Bay and in the northern part of the Nile plume. Investigation of such core material will provide information about the rate of eutrophication and might shed light on the potential natural and anthropogenic

influenced mechanisms that force changes in the trophic state of the area. This is of vital importance for policy makers in Egypt which become increasingly aware of the problems caused by eutrophication and its social-economic effects.

3- Dinoflagellate cysts have proven useful in revealing the history of eutrophication in the proximal area of Po-river discharge (Chapter 4). The mechanisms controlling these signals are however not fully understood, especially the rate in which natural and anthropogenic factors induce the observed changes. A way to obtain insight in this is to study the areas with similar climatic conditions, and thus comparable natural assemblages, but with different levels of human influence.

4- The observed dinoflagellate cyst signals in chapter 4 could also be tested against other signals both from other microfossil groups and geochemical approaches in the same core to resolve a closer cause-effect relationship, and if possible to give some quantitative measures in the study area. Therefore, multi-proxy approaches are needed in the future studies.

5- The present study differentiated between polluted and non-polluted environment in Abu-Qir Bay based on foraminiferal community (Chapter 3). It showed that the extent to which population was found to be impoverished corresponded to the degree to which the sediment was contaminated in Abu-Qir Bay. In this contaminated environment, foraminiferal tests were less diversified and aberrant tests were frequently found. It is believed that morphological abnormalities detected from Abu-Qir Bay are caused by stressed conditions on the sea bottom related to heavy metal contamination. Nevertheless, to eliminate the effect of natural environmental stresses as a possible reason for test deformities, X-ray analyses of living deformed and non-deformed individuals are needed in future.

6- Changes in foraminiferal community and the presence or absence of indicator species were excellent environmental and pollution indicator, however combining this tool with analysing the chemical composition of their tests, using the ratio of carbon and oxygen isotope will provide us with valuable palaeo-environmental information.

Appendix

Eutrophication stress on the phytoplankton community of the Abu-Qir Bay, southeastern Mediterranean Sea, a dinoflagellate perspective

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Abstract

This study is carried out on the qualitative and quantitative characteristics of phytoplankton community structure and the environmental factors that affect its distribution and the changes in Abu-Qir Bay, which is an eutrophic polluted bay in the Southeastern Mediterranean Sea (Alexandria, Egypt). The water samples were collected seasonally between May 2005 and February 2006 from the 9 sampling sites that represent different pollution sources and levels. Cluster analysis and Redundancy Analyses (RDA) were carried out to quantify the relationship between the distribution of phytoplankton community and different environmental parameters such as: heavy metal concentration, temperature, salinity, nutrient concentration, chlorophyll-*a,* dissolved oxygen, pH, and transparency of the upper water. In general, the most dominant groups are Bacillariophyceae, freshwater algae, and Dinophyceae. The bay is characterized by dinoflagellate (mainly *Gymnodinium* spp.) dominated bloom in summer, while in winter diatoms are dominant. *Gymnodinium* spp. favoured by the warm saline stratified condition in summer. Multivariate cluster analysis shows two clusters associated mainly with temperature, nutrient, salinity, and transparency. The first cluster comprises mainly diatoms, freshwater algae, and *Prorocentrum* dinoflagellate species. The second cluster is dominated by dinoflagellate. RDA analysis confirms phytoplankton community composition to be sensitive mainly to temperature, followed by nutrient concentrations, dissolved oxygen, heavy metals concentration, transparency, pH, and salinity. The positive relationship between all phytoplankton groups, except for dinoflagellate, and the nutrient is recorded. Freshwater algae are favoured by reduced salinity. The massive increase of phytoplankton standing crop, reduced diversity, frequent occurrence of toxic dinoflagellate harmful species, and the concurrent pattern between the phytoplankton and zooplankton cycles could be used as indication of water deterioration in Abu-Qir Bay. Dinoflagellate production is affected negatively with heavy metal pollution, which is expressed by its reduced abundance in the polluted station. The eurythermal euryhaline *Prorocentrum minimum* is adapted to low salinity and temperature, but high nutrient environment. *Prorocentrum* species, mainly *P. micans* and *P. triestinum*, could be used as indicator for eutrophicated polluted area, while *Ceratium* spp. could be considered as sensitive species.

1. Introduction

Eutrophication as a result of anthropogenic input of nutrients has been reported in many aquatic systems (Smith et al., 1999). In many coastal waters, increasing nutrient enrichment due to disproportionate inputs of nutrients has been shown to profoundly affect the phytoplankton species composition and production, and thus the ecosystem structure and function (Smith et al., 1999). Many nutrients may potentially limit algal growth and accumulation in aquatic systems (Fisher et al., 1999). In the last 50 years the increasing eutrophication of seas as a result of human impact has become a serious environmental problem in the world. Increased N loading has been associated with eutrophication and declines in water quality, symptoms of which include harmful algal blooms, hypoxia and anoxia events, fish kills and trophic disruption (Rosenberg, 1985; Pearl, 1997; Piehler et al., 2004). Phytoplankton constitutes an important part of the primary production in the seas. It has been well documented that initial changes in aquatic communities due to increasing eutrophication begin with the successions in the species composition and abundance of phytoplankton (Ignatiades et al., 1985; Danilov and Ekelund, 1999). Thus the information obtained from phytoplankton communities can significantly contribute to assessing eutrophication levels in aquatic areas.

Abu-Qir Bay is a shallow semi-closed basin laying about 20 km east to Alexandria city, between longitudes 30° 03' and 30° 22' E and latitudes 31° 16' and 31° 28' N. It is bordered at the north-eastern side by the Rosetta mouth of the Nile and at the south-western side by Abu-Qir head land, which recently was extended further seaward through the construction of Abu-Qir Harbor. The bay occupies an area of 500-600 km^2 with average depth of 10-12 m. It represents one of the unique coastal ecosystems that subject to several land-based sources, like freshwater from the Rosetta mouth of the Nile, loaded by nutrients, Lake Edku effluent carried with trace metals, pesticides, humic acids and nutrients, and El-Tabia Pumping Station which pours industrial and domestic wastes. The bay is also exposed to oil pollution from fishing boats, the activities of gas production liquefying, and export field, in addition to the activities of Abu-Qir Fertilizers Company, and Abu-Qir Electrical Power Station.

Abu-Qir Bay represents a considerable source of fish production in Egypt. The role of phytoplankton as the main source of food supply for fish is well known, thus it is necessary to study the standing crop of dinoflagellate as a part of phytoplankton in Abu-Qir Bay. Previous studies which have focused on Abu-Qir Bay are related to physico- chemical characteristics of water (Dowidar et al., 1983; Osman and Dorgham, 1987; El-Gindy, 1988; El-Gindy et al., 1988; Nessim and EL-Deek, 1993; and Abdel Aziz et al., 2001). However, the plankton as an important biological component has attracted little attention, particularly phytoplankton. A few studies were conducted on phytoplankton in Abu-Qir Bay (Dowidar et al., 1983; Dorgham and Osman, 1987; Samaan and Mikhail, 1990; ElSherif and Gharib, 1994; ElSherif and Mikhail, 2003; Shams El-Din and Dorgham, 2007). Very few studies concerned with the effect of pollution on phytoplankton especially dinoflagellate in this bay (Ismael, 1998).

The main objective of this work which was carried out between May 2005 and February 2006, is to examine seasonal and regional patterns in phytoplankton communities, and to study the influence of chemical-physical, trophic, and pollution factors on the phytoplankton communities, with special focus on dinoflagellate in Abu-Qir Bay (south-eastern Mediterranean Sea) on a seasonally time scale. Nine stations were sampled seasonally close to different level of pollution sources. Dinoflagellate species were related to environmental variables for better understanding of the dinoflagellate group trend in this area of the Mediterranean Sea which is strongly influenced by eutrophication and pollution.

2. Material and method

Sampling locations in Abu-Qir Bay (Fig. 1) were selected to cover different conditions resulted from different sources and levels of pollution according to table 1. Phytoplankton and water samples were collected in four seasons from May 2005 to February 2006 in the nine stations as shown in Fig. (1). During the field study, the physical and chemical parameters that affect the phytoplankton were measured. Transparency was determined with a Secchi disk. Water temperature (with a thermometer), pH (with a pH meter), salinity (with salinometer) and dissolved oxygen (DO) (Winkler method) were measured. Samples for the analysis of nutrient (nitrate, phosphate, and silicate) were deep frozen to -20 °C and stored not longer

than two weeks. Water samples were filtered before nutrient analyses. Analyses of the dissolved inorganic nutrients were performed following the method of Strickland and Parsons (1972). For quantification of chlorophyll-*a* (Chl-*a*), sub samples were filtered on 0.45 mm membrane filters (Sartorius). The Chl-*a* was extracted in 10 ml 90% acetone for 18-24 h, in the dark at ±4 °C, and the extract concentration analyzed spectrophotometrically according to (APHA, 1985). Eleven heavy metals, lead (Pb), zinc (Zn), iron (Fe), copper (Cu), nickel (Ni), chromium (Cr), cadmium (Cd), manganese (Mn), vanidium (V), cobalt (Co), and Scandium (Sc), were measured using the Inductively coupled plasma mass spectroscopy (ICP-MS).

Plankton samples were collected using water bottle 1L. The samples were preserved in 20 ml formalin (4%) in the field. Lugol´s solution was used to stain the samples. Sedimentation was occurred by standing the phytoplankton samples over night (24 hr) in 1 L cylinder. The siphonation was carried out to concentrate the phytoplankton samples to 100 ml. The subsample one ml each was transferred into Rafter counting cell. The samples were examined in Rafter cell and were counted using an inverted microscope. Plankton samples were counted as cells per liter.

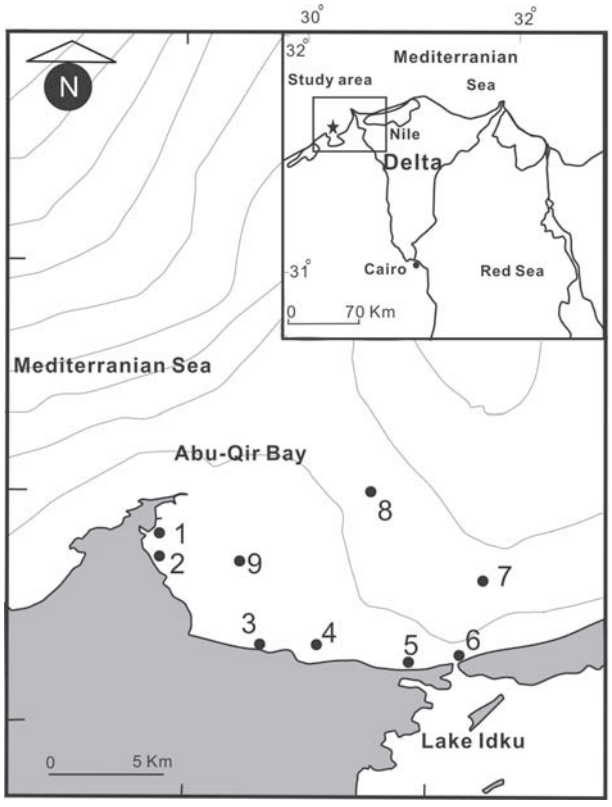

Figure 1. Study area and location of sampling stations.

Table 1. Location and description of the studied stations.

Stations	Average depths (m)	Latitude	Longitude	Location and description
St.1	3.38	31° 19′ 068″	30° 04′ 285″	In front of Abu-Qir harbor.
St.2	0.83	31° 18′ 595″	30° 04′ 314″	Located to the south of the harbor, it is called Dead Sea.
St.3	1.15	31 ° 16′ 666″	30 ° 06′ 432″	Immediately down stream from El-Tabia pumping station, occasionally exposed to the effluent water.
St.4	1.81	31° 16′ 631″	30 ° 07′ 716″	In front of Abu-Qir Fertilizer Company.
St.5	3.38	31° 16′ 363″	30 ° 09′ 617″	In front of the Petroject Company.
St.6	4.13	31° 16′ 35″	30° 10′ 20″	In front of the lake- sea channel (Boughaz El-Maadia), this station is mostly exposed to brackish water.
St.7	8.25	31° 18′ 45″	30 ° 11′ 15″	Located offshore from St.6 and to the east of it.
St.8	8.88	31° 20′ 05″	30 ° 09′ 25″	Located offshore from St.7 and St.9. It is the most offshore station, it receives no discharge.
St.9	4.63	31° 18′ 029″	30 ° 06′ 140″	Located offshore from El-Tabia pumping station

3. Data analysis

In order to correlate the different phytoplankton group and the different environmental parameters to each other, a linear correlation analysis was computed. R-mode cluster analysis was used to derive different types of phytoplankton communities especially dinoflagellate. The analysis was applied on the data comprising the grouping of all diatoms as one group, and grouping of Chlorophycea, Cyanophycea, and Euglenophycea as Freshwater algae, while no grouping of different dinoflagellate taxa were computed in the analysis. The analysis was performed on the most dominant dinoflagellate species (*Gymnodinium* spp., *Prorocentrum* spp., *Protoperidinium* spp., *Gonyaulax* spp., *Pyrophacus* spp., *Gyrodinium* spp., *Oxytoxum* spp., and *Amphidinium* spp. Within genus *Prorocentrum,* eleven species were included in the analysis (*P. arcuatum*, *P. belizeanus, P. compressum, P. concavum, P. gracile, P. lima, P. mexicanum, P. micans P. minimum, P. scutellum,* and *P. triestinum*). The clusters were developed from similarity matrices calculated using correlation coefficient. The previous analyses were carried out by PAST Palaeontological Statistics Program, version 1.88.

To better evaluate the nature of the recognized species, Fisher's alpha diversity index was estimated by PAST program as the following: Fisher's alpha index is defined

implicitly by the formula $S = \alpha \ln (1 + n / \alpha)$ where S is number of taxa, n is number of individuals, and α is the Fisher's alpha.

Multivariate redundancy analysis (RDA) was applied as a tool to evaluate the relationships between environmental and biological variables. Because the length of gradient is less than 2, which indicate the linear distribution, RDA was chosen. This method is normally used in ecology for ordination by direct gradient analysis, where a matrix of species variables is analysed with regard to a corresponding matrix of environmental variables (see Legendre and Anderson, 1999). Monte Carlo permutation test was applied in order to rank the explanatory variables according to their importance. The significance of the single environmental variables was statistically tested by this test with 199 permutations. Covariance between environmental variables was determined and corrected by forward selection of the single parameters. For means of interpretation, the first two axes were portrayed as biplots and the variance explained by each significant explanatory variable was tabulated. This analysis was calculated by means of CANOCO program, version 4.02.

4. Results

4.1. Environmental parameters

The surface water temperature has marked seasonal variations in Abu-Qir Bay; the maximum and the minimum surface water temperatures are 32.5° C during the summer and 18° C during the winter. Concerning the spatial variations, St.4 (Abu-Qir Fertilizer Company) shows the highest water temperature (Fig. 2).

Distribution of salinity in Abu-Qir Bay is generally a result of interaction between sea water with brackish and polluted waters, discharged into this bay via El-Maadia outlet, El-Tabia pumping station, and some companies (e.g. Abu-Qir Fertilizer Company). This is confirmed by the low surface salinity at St.3, St.4, and St.6 (Fig. 2). Furthermore the high differences between the bottom and the surface salinity during the investigated period reflect the strong effect of the intrusion of fresh and brackish waters especially from El-Maadia channel, El-Tabia pumping station, and Abu-Qir Fertilizer Company (Appendix 1).

The pH in Abu-Qir Bay during the period of study is found to be on the alkaline side, giving an annual mean of 8.06 for the water column. The regional pH values vary between a minimum of 7.27 at St.3 (El-Tabia pumping station) and a maximum of 9.04 at St.6 (El-Maadia) (Fig. 2). This is most probably due to the effect of the industrial discharge at St.3, which is enriched with organic matter, while due to the increase in photosynthetic activity and the corresponding increase in the dissolved oxygen concentration at St.6. The distribution of pH is positively correlated with total phytoplankton density ($r = 0.55$, $p \leq 0.05$) and DO concentration ($r = 0.82$, $P \leq 0.05$) indicating that pH may also reflect the redox potential of this area. The seasonal variation of pH values generally is very slight.

The concentration of DO in Abu-Qir bay varies markedly from zero to 7.99 mg/L. Regarding the regional dissolved oxygen distribution; St.3, followed by St.4 show pronounced decrease in the dissolved oxygen concentration (reach anoxic condition) (Fig. 2). This is due to mainly the highest amount of industrial wastes discharged from El-Tabia pumping station which contained more reducible material (i.e., this organic discharged material attributed to the consumption of DO). Station 6 is characterized by the highest dissolved oxygen concentration (7.99 mg/L) (Fig. 2). This is coincided mainly with the increased photosynthetic activities of phytoplankton abundance in this station. Seasonally, February has more or less the highest DO concentration, which coincides mainly with strong currents and water turbulence dominated in the cold season, beside the increase in DO solution by lowering temperature. The negative correlation between temperature and DO concentration is recorded ($r = -0.65$, $P \leq 0.05$)

The highest Chl-*a* value (48.25µg/L) is recorded at St.6 in February, while the lowest values are recorded at St.8 and St.7 in August (Fig. 2). This reflects the influence of nutrient enriched water from Lake Edku which enhances primary productivity. The recorded data show that the photosynthetic potential decreases markedly towards the offshore stations.

The Secchi value is markedly low at St.3 especially in February (reached to 0 m). The highest values of transparency are recorded at St.8 and St.7 in May (3 m) (Fig. 2). This is due to the suspended silt particles stirred up by the water currents, as well as to the nature of the discharged water through El-Tabia pumping station, which is

originally turbid and consequently attributed to the shallowness of El-Tabia region. On the other hand, it is clear that transparency increases seaward and decreases near the entrance of the drains. The calm weather in May leads to increasing of the water transparency in this month, while the prevailing wind in winter leads to stirring up of the bottom sediments, increasing the amount of suspended matter, and decreasing transparency.

Concerning nutrient concentrations, the horizontal distribution of the reactive phosphate in Abu-Qir Bay shows that the highest concentration is recorded at St.6 (17.5 µg at/L), followed by St.4 and St.3 (Fig. 3). The nitrate concentration follows the same trend of increasing values at St.6 and St.3 but with a very pronounced peak at St.4. Concerning the silicate concentration, St.4 and St.3 are more enriched than St.6. This reflects the direct effects of the lake water discharges originated mainly from agricultural run-off and run off from Abu-Qir Fertilizer Company (allochtonous source) in increasing the nutrient level. On the other hand, the lowest values are observed at St.8 or/and St.7, which reflects the position of this reference station faraway from pollution sources supplemented by high nutrient (Fig. 3).

Geochemical results show high concentrations for all the measured heavy metals. The maximum concentrations of most of the measured heavy metals (e.g., Cd, Sc, V, Cr, Ni, Cu, and Zn) are recorded in St.2 (Dead Sea), followed by St.1 (harbor), St.3 (in the vicinity of El-Tabia pumping station), St.6 (in the vicinity of Boughaz El-Maadia), and St.4 (in the vicinity of Abu-Qir Fertilizer Company), with few exceptions (Fig. 4). Station 6 has a pronounced increase of Mn, Cr, V, and Co. Zinc tends to increase in St.3 and St.4 whereas Mn, Fe, Co, and Pb show increase in St.1 (Fig. 4).

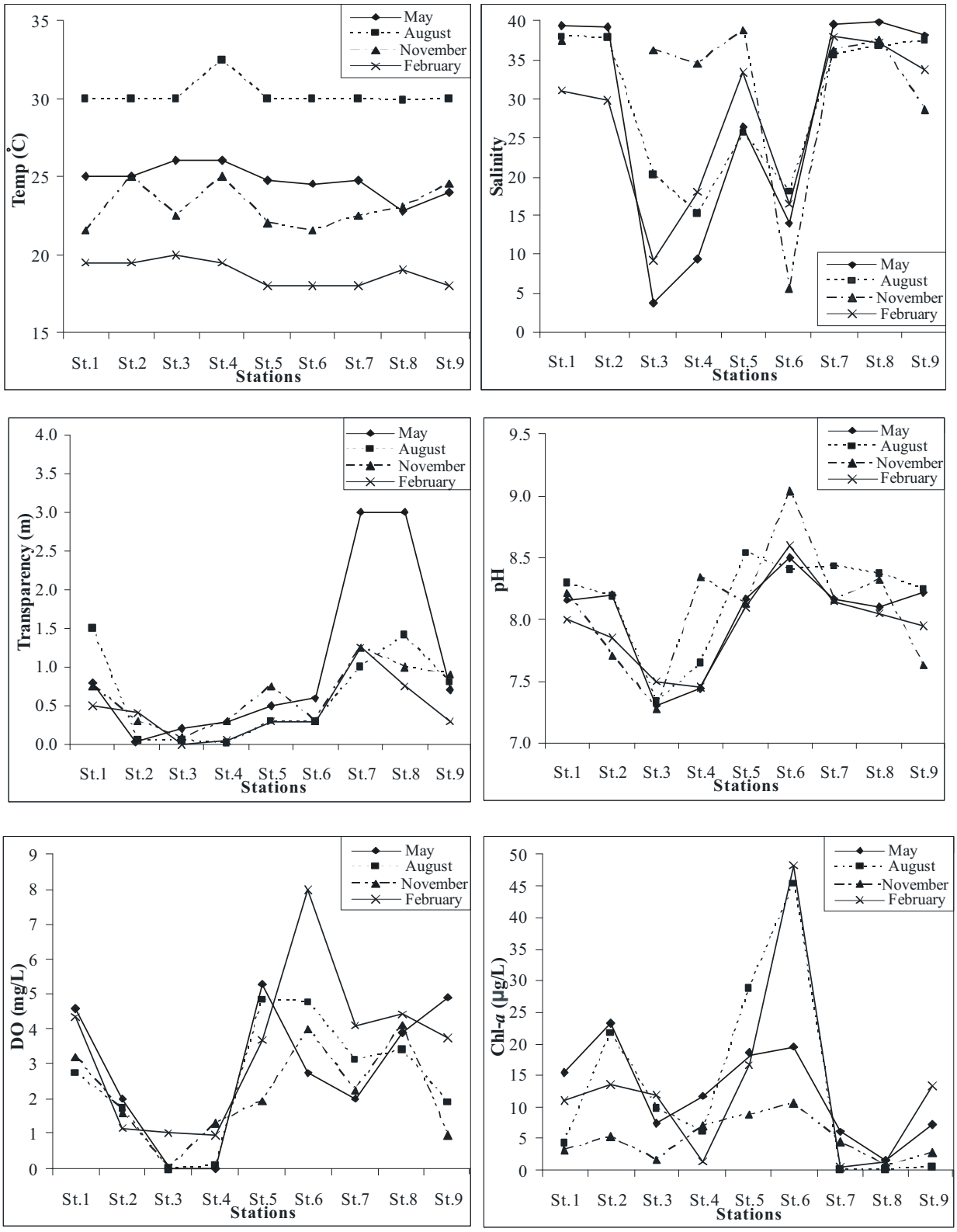

Figure 2. Seasonal and spatial variations of surface water temperature (°C), salinity (‰), transparency (m), pH, Chl-*a* (µg/L), and DO (mg/L) in Abu-Qir Bay during 2005 - 2006.

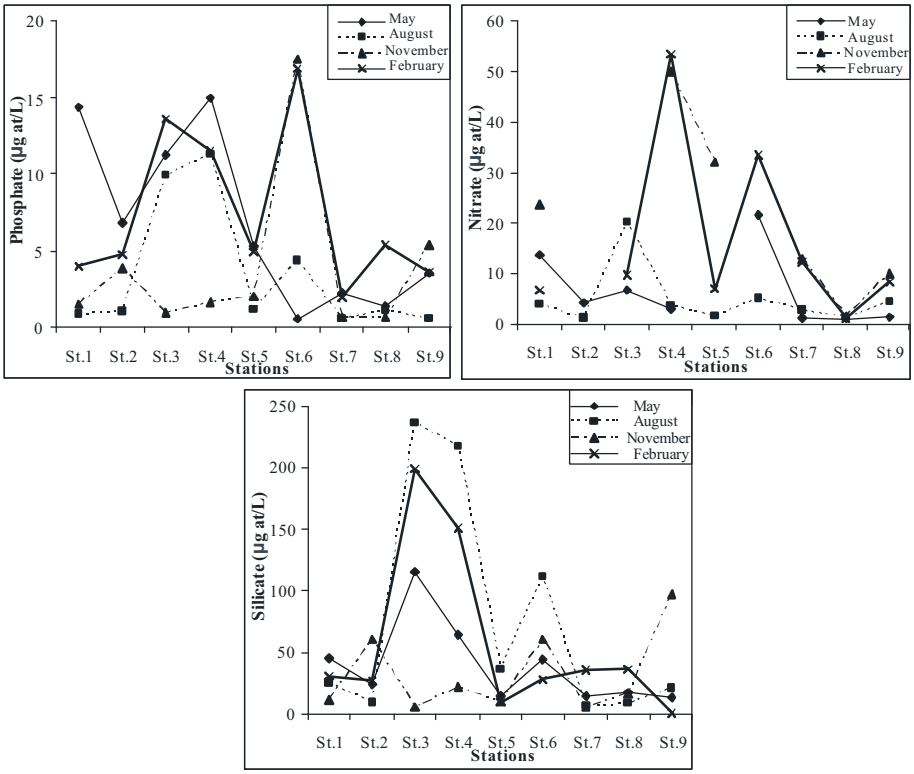

Figure 3. Seasonal and spatial variations of nitrate (µg at/L), phosphate (µg at/L), and silicate (µg at/L) in Abu-Qir Bay during 2005 – 2006.

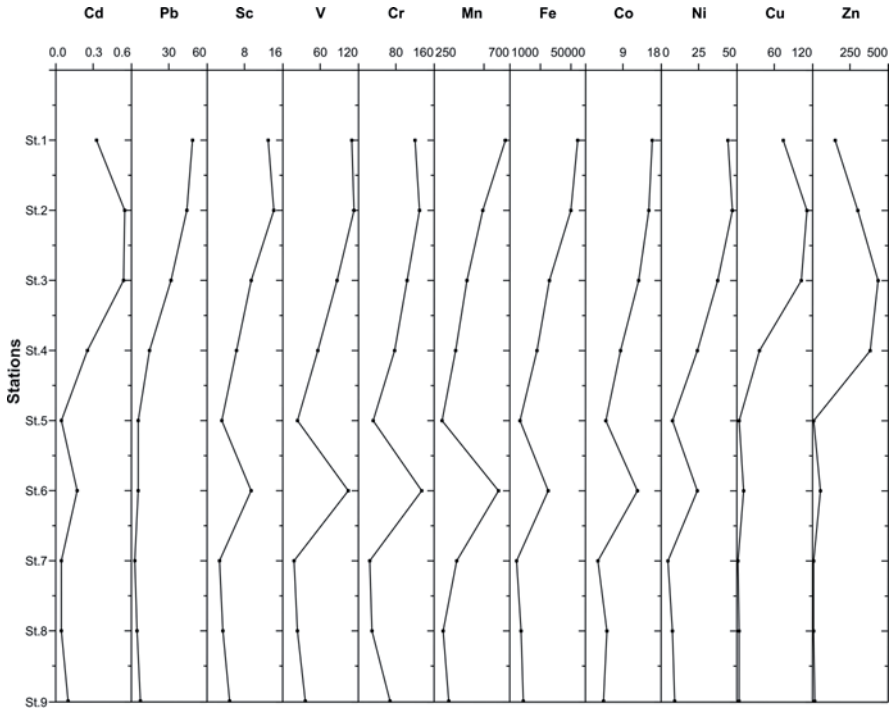

Figure 4. Heavy metal concentrations (ppm) of the surface sediment in Abu-Qir Bay. The scale of all the parameters begin from 0 value except for Mn (250 ppm) and Fe (1000 ppm).

4.2. The phytoplankton population

4.2.1. Phytoplankton community composition, standing crop, and diversity

The major components of phytoplankton community during the study period are diatoms, chlorophytes, dinoflagellates, cyanophytes, and euglenophytes. Silicoflagellates are represented by only one species; *Dictyocha fibula*. A total of 100 taxa were recorded during the present study, comprising diatoms (21), dinoflagellates (47), chlorophytes (17), cyanophytes (8), euglenophytes (6), and silicoflagellates (1). Bacillariophyceae dominates the other phytoplankton components, and formed about 78.8% of the total phytoplankton. Freshwater algae (FW) contribute about 16.2% to the total phytoplankton (9.3% chlorophytes, 5% euglenophytes, 1.9% cyanophytes, and 0.02% silicoflagellates), while dinophyceae contributes 5% (Fig. 5).

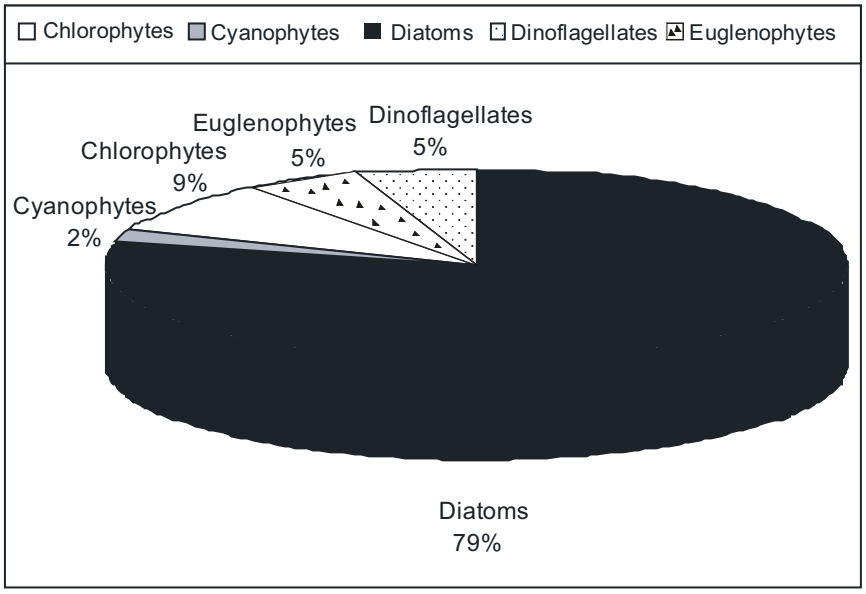

Figure 5. Relative contribution of the different phytoplankton groups in Abu-Qir Bay during the period of study.

Compared to the previous studies occurred on phytoplankton in Abu-Qir Bay, the surface phytoplankton standing crop proves to be comparatively rich. The maximum phytoplankton standing crop is $13*10^5$ cell L^{-1} (at St.6, February) (Fig. 6). It is much higher by about 28 folds than that previously recorded at the same region during 1998 (Ismael, 1998). Ismael (1998) reported that the maximum phytoplankton

standing crop didn't exceed $45.7*10^3$ cell L^{-1}. In addition, Elsherif and Gharib (1994) found even a lower crop than Ismael (1998). This is related to the increase of eutrophication state in the bay especially at St.6.

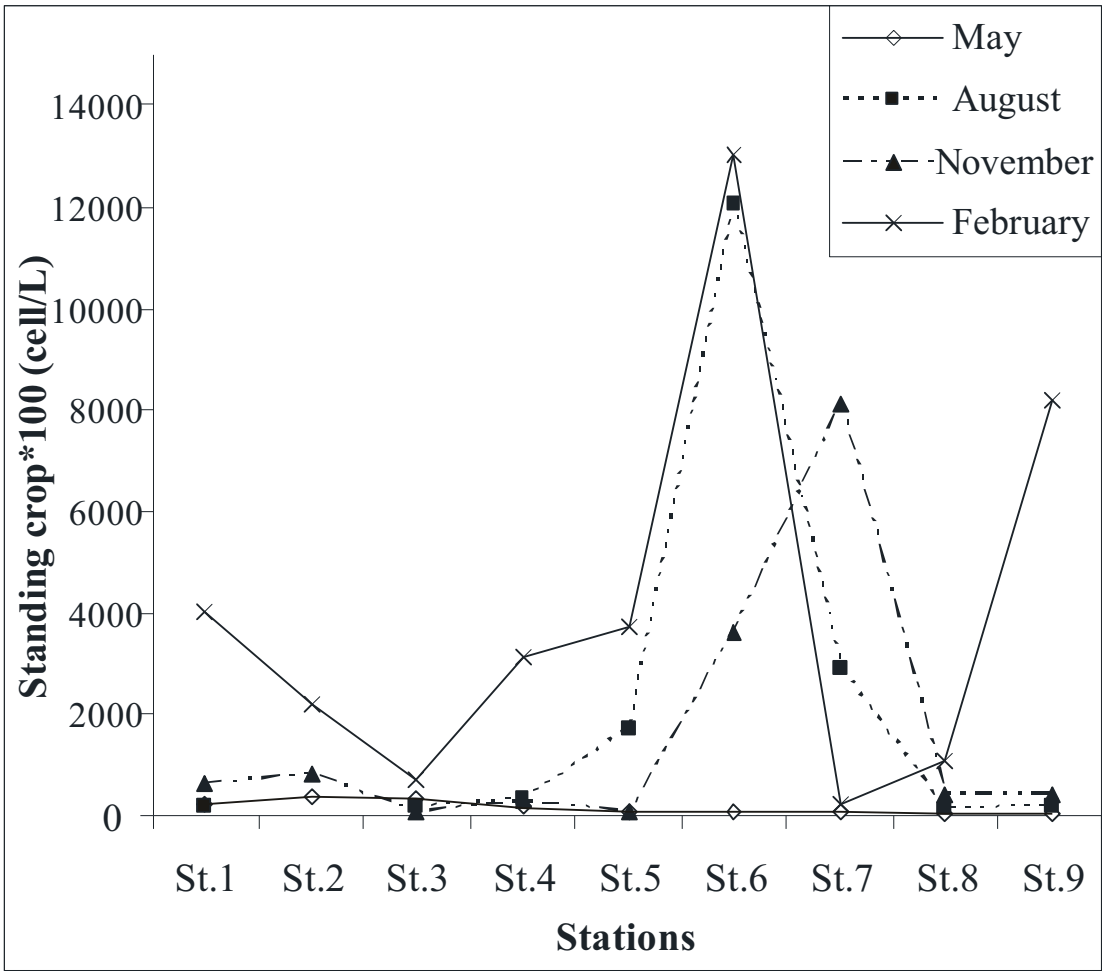

Figure 6. Seasonal and spatial variations of the total phytoplankton (cell/L) in Abu-Qir Bay during the study period.

Abu-Qir Bay is characterized by low diversity as indicated by Fisher's alpha diversity index. It ranges between 2.05 at St.6 (November) and 10.00 at St.8 (May), with the exception of higher value of 13.93 at St.8 (November). Station 6 is the least diversified station. The diversity increases in offshore less polluted stations (e.g. St.8 and St.9) (Fig. 7).

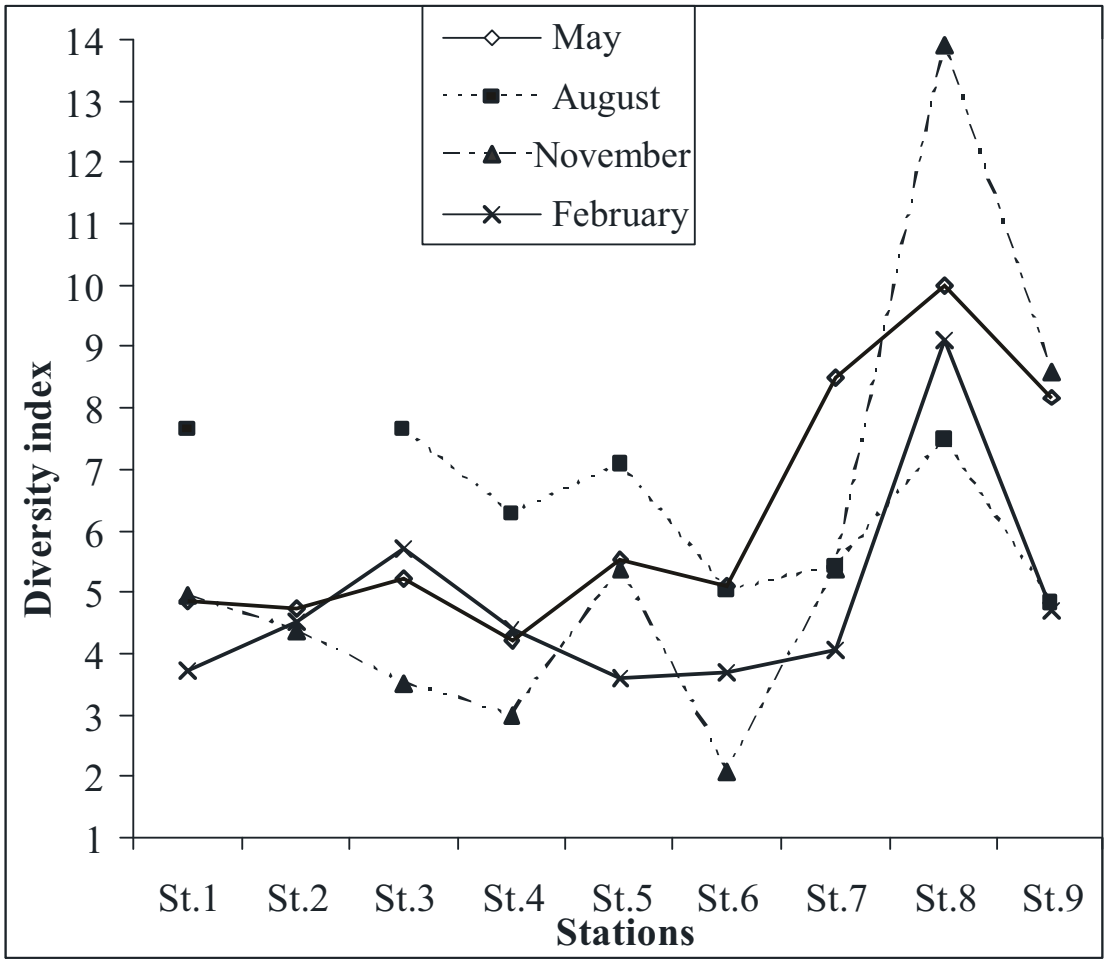

Figure 7. Seasonal and spatial variations of Fisher's alpha diversity index in Abu-Qir Bay during the period of study.

4.2.2. Seasonal and spatial variations of plankton community

The total phytoplankton standing crop shows a high pronounced peak in winter 2006. It comprises mainly diatoms which show maximum persistence in winter season (Fig. 8). We find a negative significant correlation between phytoplankton standing crop and water temperature ($r = -0.62$, $P \leq 0.05$). The lowest phytoplankton standing crop is recorded during May 2005. There are minimum values of nearly all groups in this month. On the contrary, dinoflagellate is the only group which has an outstanding peak during summer season 2005 (Fig. 8). Therefore, periods of maximum dinoflagellate cell numbers are generally altered with periods dominated by diatoms. The respective quantitative cycles of the total phytoplankton standing

crop and that of diatoms, dinoflagellate, freshwater algae, and of zooplankton can be compared as shown in figure (8).

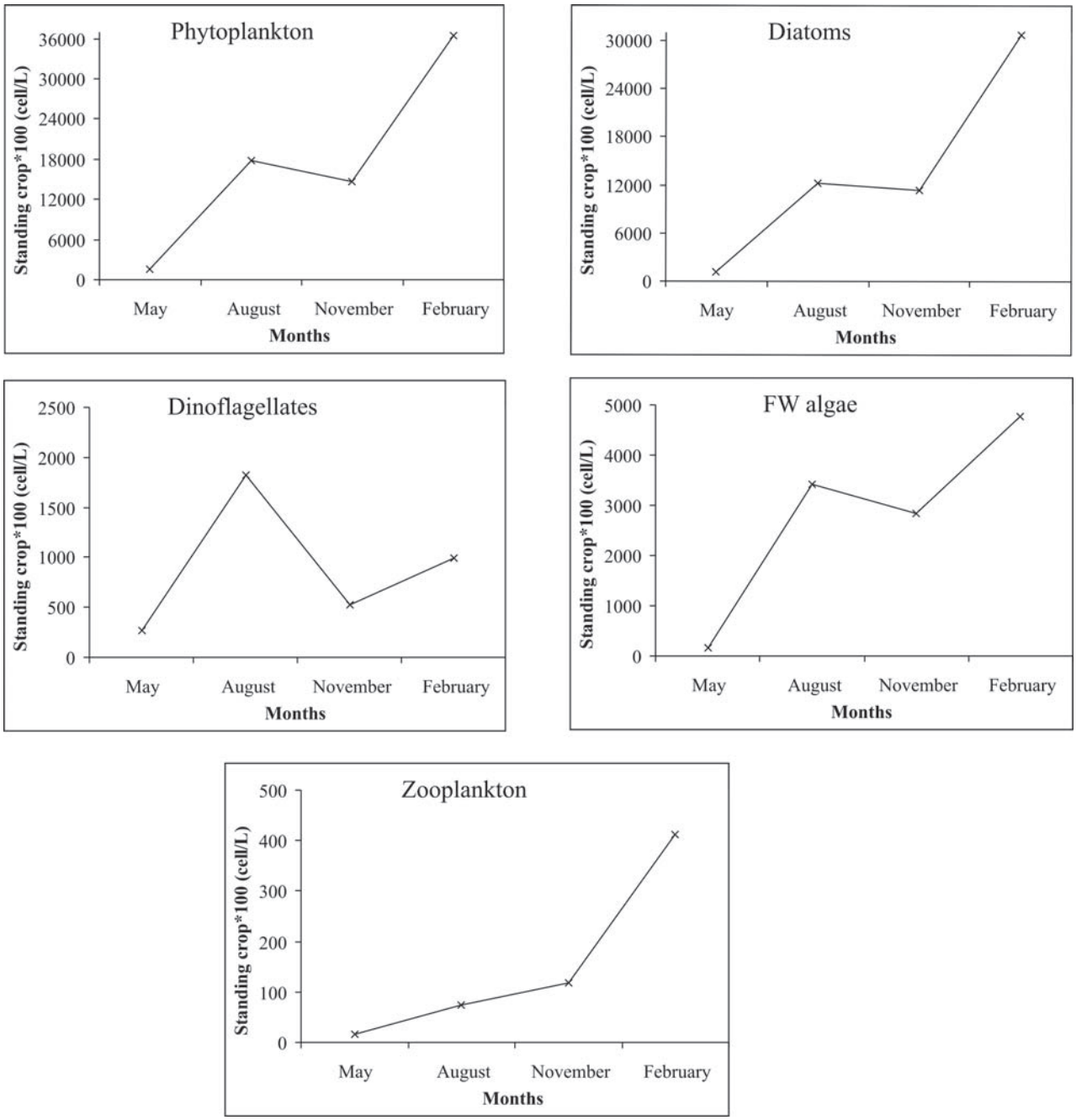

Figure 8. Seasonal variations of the different groups of plankton in Abu-Qir Bay during the period of study.

With regard to the phytoplankton spatial distribution, it varies greatly from station to another. Abu-Qir Bay sustains a heavy growth of phytoplankton at some stations especially St.6 (in front of Bougas El-Maadia) ($288*10^4$ cell L^{-1}) as a result of

continuous discharge of allocthonous nutrient and brackish water from Lake Edku. This is associated with the high pH value and high dissolved oxygen concentration at this station. The total phytoplankton abundance is significantly positively correlated with pH and DO values (r = 0.55 and 0.82 respectively at $P \leq 0.05$). The highest phytoplankton counts of St.6 are due to the dominance of diatoms, mainly *Cyclotella* spp., *Melosira* spp., and *Nitschia* spp., and chlorophyta. El-Tabia pumping station (St.3) has the lowest total phytoplankton standing crop ($14*10^4$ cell L^{-1}) (Fig. 9). This is related to the sharp decrease in the number of diatoms.

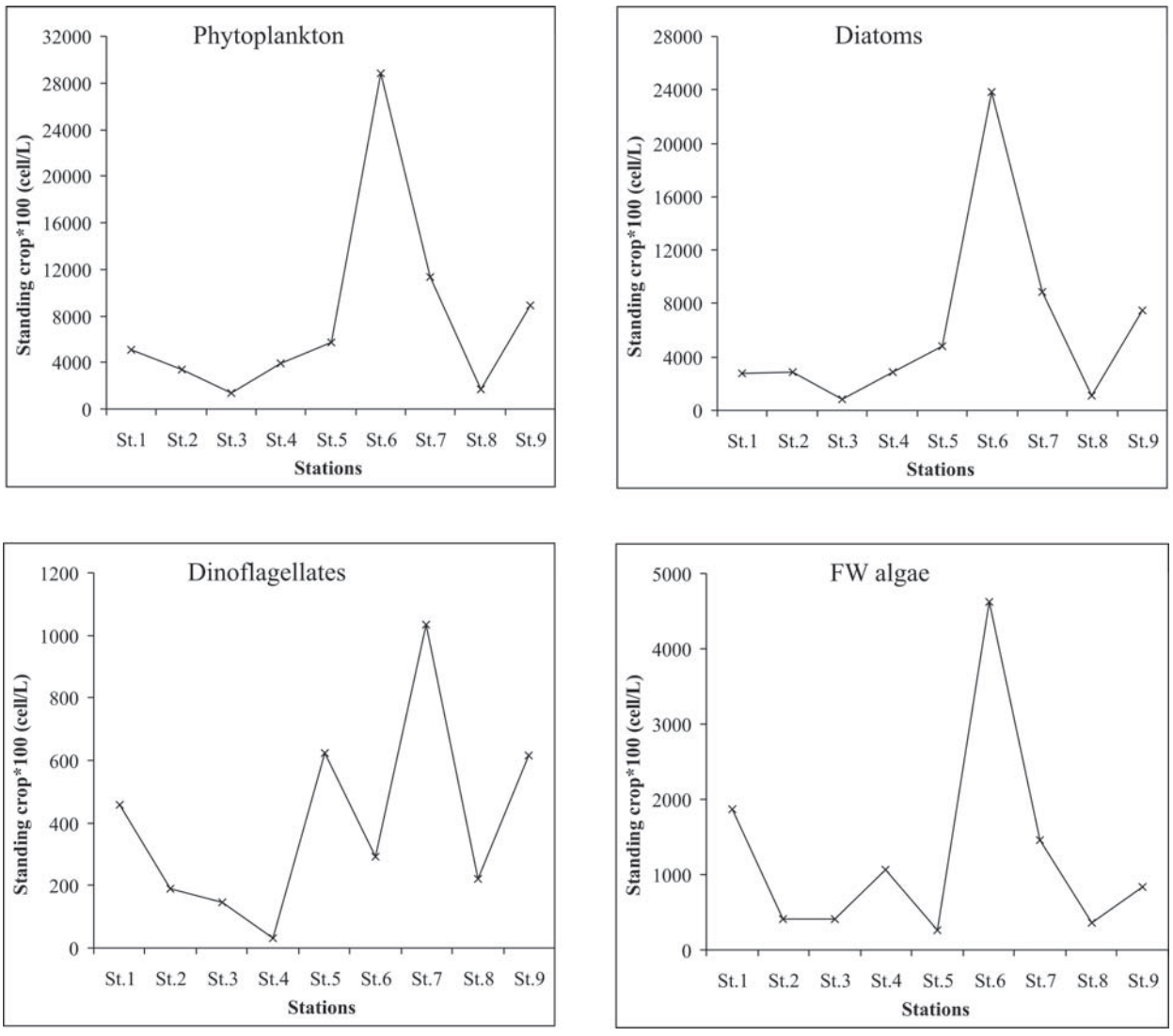

Figure 9. Spatial variations of the different groups of phytoplankton in Abu-Qir Bay during the period of study.

The zooplankton cycle shows the same seasonal pattern as phytoplankton. The maximum abundance is recorded in February, while the minimum abundance is

recorded in May (Fig. 8). The zooplankton population is dominated by Tintinnids and Rotifers.

4.3. Dinoflagellate population

4.3.1. Dinoflagellate community composition

During the present study, 47 dinoflagellate taxa are recorded. Although the dinoflagellate community of Abu-Qir Bay is characterized by the presence of relatively large number of taxa, few of them are responsible for the bulk of the population. There are 3 taxa represent about 75% of the total dinoflagellate community. *Gymnodinium* spp. is the most dominant species, contributing 36.7% to the total dinoflagellate community, followed by the genus *Prorocentrum* (24.1%), and *Protoperidinium* spp. (13.8%). *Gonyaulax* spp. is the fourth dominant species; its percentage composition is 5.4% of the total dinoflagellates community. Genus *Prorocentrum* is very diversified in the study area (11 species), with the dominance of *P. micans* in most stations.

The different dinoflagellate taxa detected show different periods of permanence in the ecosystem studied. The genus *Prorocentrum* is dominant in February (46.13%) and in May (37%) (Fig.10). *Prorocentrum micans* is the leading species in February. *Prorocentrum micans, P. gracile*, and *P. triestinum* are the most three dominant species in May. In August, the bulk of dinoflagellate comprises *Gymnodinium* spp (53.88%). This unarmoured non toxic planktonic species forms bloom in this season. In November, *Protoperidinium* spp. (25.1%), *Gymnodinium* spp. (22.8%), and *Prorocentrum* spp. (17.8%) have nearly the same contribution to the total dinoflagellates community (Fig. 10). Dinoflagellate community structure varies greatly among different stations. Genus *Prorocentrum* dominates the highly eutrophicated stations (St.6, St.3, St.4, and St.9). Station 5 and St.7 are subjected to *Gymnodinium* spp. bloom (63%, and 44.6% of the total dinoflagellates community) (Fig. 11). Station 1 and St.2 are dominated by *Gymnodinium* spp., *Protoperidinium* spp., and followed by *Gonyaulax* spp.

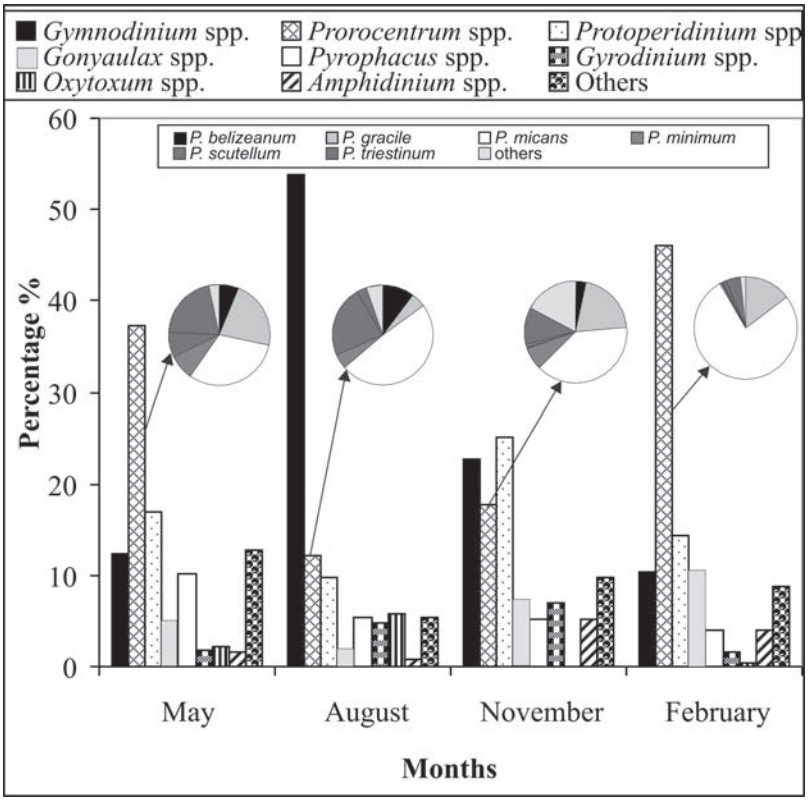

Figure 10. Seasonal relative contribution of the most dominant dinoflagellate species and the percentage composition of different prorocentrum species in Abu-Qir bay.

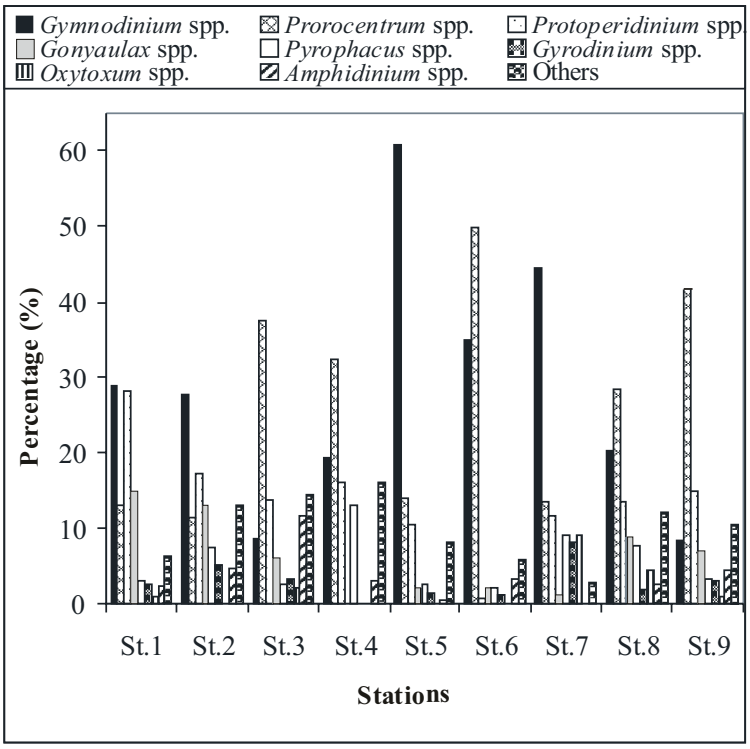

Figure 11. Spatial percentage composition of the most dominant dinoflagellate species in Abu-Qir Bay during the study period.

4.3.2. Temporal and spatial distribution of dinoflagellate community

The standing crop and the primary production of the dinoflagellate show pronounced variations at different stations and months correlated with physico-chemical conditions of water. Throughout the sampling period, August 2005 is the month in which dinoflagellate reaches its maximal standing crop values ($89.7*10^3$ cell L^{-1}) at St.7, followed by St.5 ($48.3*10^3$ cell L^{-1}). The minimum dinoflagellates standing crop is recorded in all seasons at St.4; it disappears completely in November at this station. Except of these extremes, the dinoflagellates standing crop has a relatively small range of variation among the different seasons and stations (Table 2 and Fig. 12). With regard to the spatial total dinoflagellate distribution among stations, dinoflagellate dominates St.7, followed by St.5, St.9, and then St.1. Station 4 has the minimal value of dinoflagellate standing crop, followed by St.3.

Table 2. Seasonal and spatial variations of total dinoflagellates abundance*100 (cell L^{-1}) in Abu-Qir Bay during the period of study.

Dinoflagellates	**May**	**August**	**November**	**February**
St.1	8	150	175	125
St.2	35	-	50	106
St.3	41	14	18	74
St.4	8	14	0	9
St.5	43	483	40	58
St.6	43	125	48	75
St.7	65	897	27	44
St.8	12	66	33	111
St.9	11	79	131	393

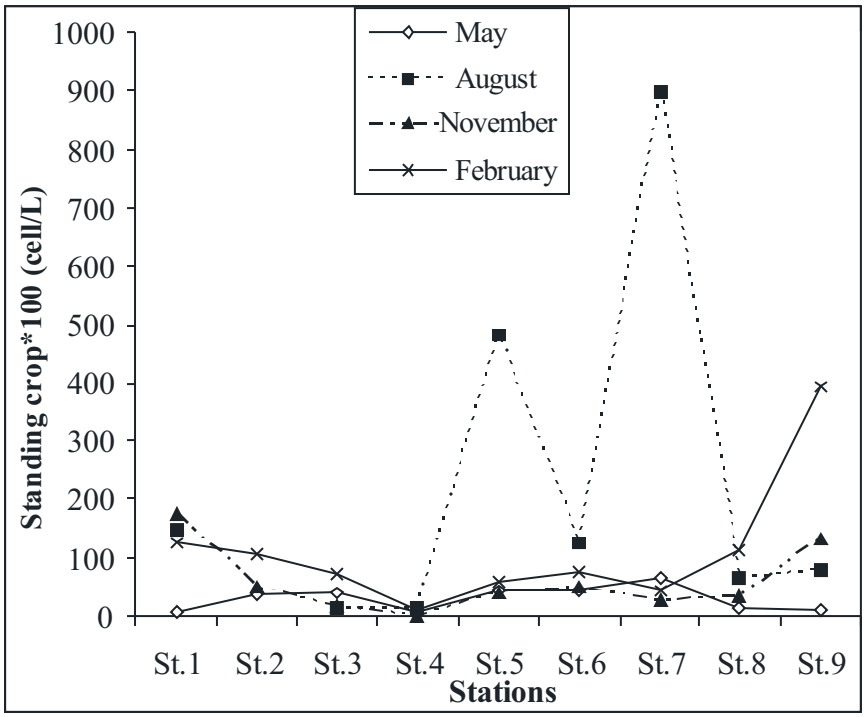

Figure 12. Seasonal and spatial variations of dinoflagellates (cell/L) in Abu-Qir Bay during the study period.

4.4. Statistical analyses

4.4.1 Cluster analysis

The R-mode cluster analysis classifies the phytoplankton communities into two main clusters. Each cluster is characterized by a particular association of phytoplankton, reflecting primarily environmental influences. Figure 13 displays the resulting dendrogram. The high proportion of diatoms, freshwater algae, *Amphidinium* spp., genus *Prorocentrum* with its entire species except *P. concavum,* and *P. lima* characterize the first cluster. This cluster indicates highly eutrophicated environment, with low temperature, low salinity, and turbid surface water. Cluster 1 is restricted to the cold season in February, especially at St.6 where it is dominanted by brackish water. The second cluster comprises warm, saline, transparent, and less eutrophied environment. The association in this cluster is bloomed mainly in the summer (August). The association is co-dominated by total dinoflagellate abundances, *Gymnodinium* spp., *Protoperidinium* spp., *Gonyaulax* spp., *Pyrophacus* spp., *Gyrodinium* spp., *Oxytoxum* spp., *P. concavum,* and *P. lima.*

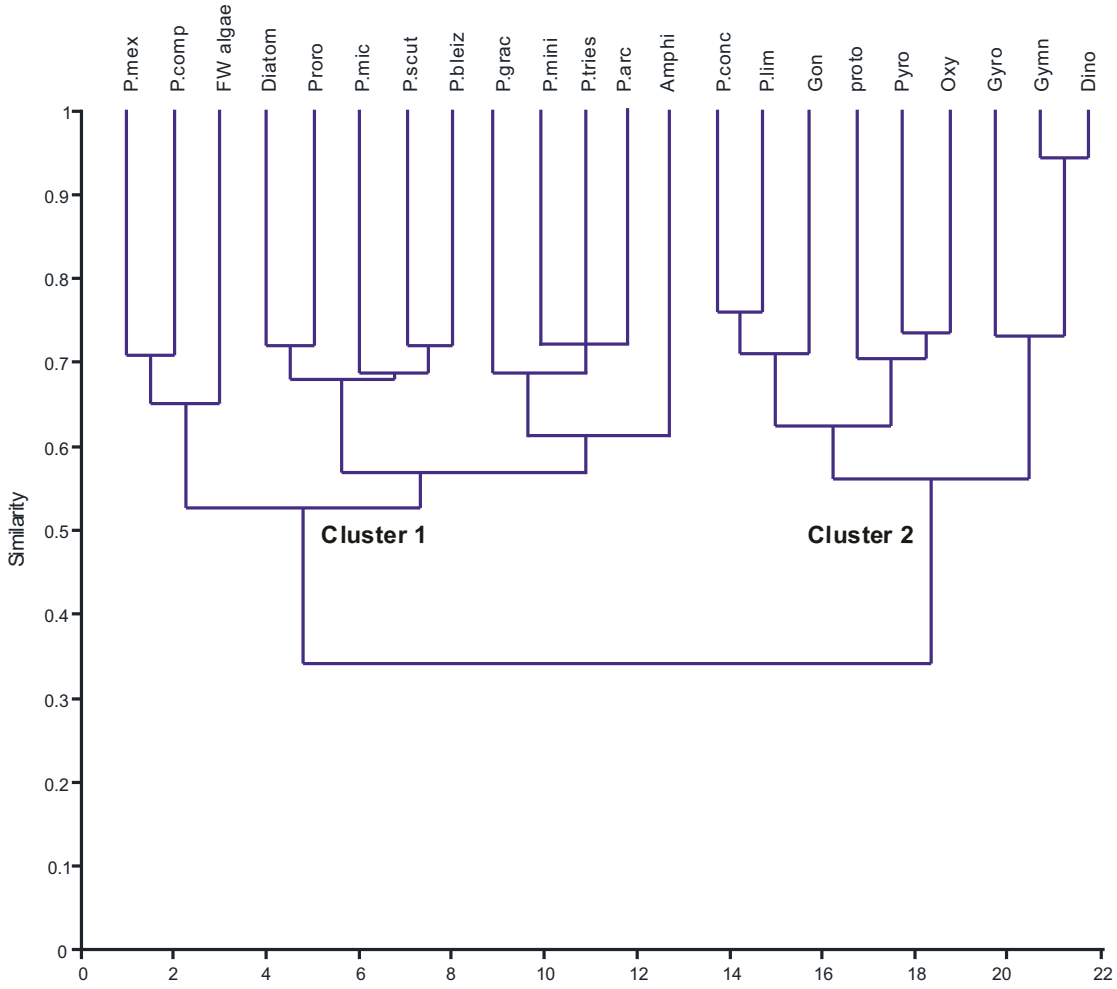

Figure 13. R-mode cluster analysis of the phytoplankton community. Correlation Coefficient was used. Abbreviations: Amphi: *Amphidinium* spp., Dino: total dinoflagellates, Gon: *Gonyaulax* spp., Gymn: *Gymnodinium* spp., Gyro: *Gyrodinium* spp., Oxy: *Oxytoxum* spp., Proro: *Prorocentrum* spp., P.arc: *P. arcuatum*, P.bleiz: *P. belizeanus*, P.comp: *P. compressum*, P.conc: *P. concavum*, P.grac: *P. gracile*, P.lim: *P. lima*, P.mex: *P. mexicanum*, P.mic: *P. micans*, P.mini: *P. minimum*, P.scut: *P. scutellum*, P.tries: *P. triestinum*, proto: *Protoperidinium* spp., Pyro: *Pyrophacus* spp.

4.4.2 Multivariate Redundancy Analysis (RDA)

The results of RDA are presented in Fig. 14 and Table 3. The first two dimensions of the RDA accounted to 73% of the total variance of phytoplankton abundances and environmental data. The percentage of variability explained by temperature is 40% (Table 3). Temperature appears to be the most significant factor shaping the phytoplankton community structure in the study area and is positively related with the total dinoflagellate abundances, *Gymnodinium* spp., and to a lesser extent with *Protoperidinium* spp., *Gonyaulax* spp., *Oxytoxum* spp., *Gyrodinium* spp., and

Pyrophacus spp., but negatively correlated with diatoms, freshwater algae, and the genus *Prorocentrum* including *P. micans, P.* scutellum*, P. minimum, P. triestinum,* and *P. compressum* (Fig. 14). Phosphate, pH, Co, Mn, DO, Chl-*a*, and nitrate go in more or less the same direction (are ordinated on the positive side of the first axis). They affect the total dinoflagellate abundances and *Gymnodinium* spp negatively, while they affect all species located at the positive side of the first axis positively (Fig. 14). The RDA analysis confirms the positive influence of silicate on the total dinoflagellate abundances, *Gymnodinium* spp., and freshwater algae. However, for diatoms, and *P. micans* there is a distinct negative influence. This negative relationship especially with diatoms suggesting that diatom is generally using up the silicate which is necessary for its frustule. A negative influence of salinity and transparency is indicated for freshwater algae, diatoms, and the genus *Prorocentrum* including *P. micans, P. compressum, P.* scutellum*, P. minimum,* and *P. triestinum*, while the positive influence is indicated for the total dinoflagellate abundances, and *Gymnodinium* spp. (Fig. 14). *Amphidinium* spp., *P. arcuatum*, *P. mexicanum, P. belizeanum, P. concavum,* and *P. Lima,* are located at the centre of the diagram, they don't show any significant relationship with the observed environmental parameters. Mn and Co are the only significant heavy metals; they show negative effect on all dinoflagelate community (except the genus *Prorocentrum*). This could explain why dinoflagelate has a remarkable low standing crop at St.3 and St.4. The other heavy metals are insignificant in the study area; since they have F values < 1.

Table 3. Fraction of variance explained by the only significant environmental variables used in RDA

Variable	LambdaA	F value for data
Temperature	0.40	5.320
Chl-*a*	0.08	3.630
DO	0.07	4.800
Nitrate	0.06	3.800
Silicate	0.05	3.890
Mn	0.04	2.820
Transparency	0.03	2.370
pH	0.03	2.370
Salinity	0.02	1.690
Phosphate	0.02	1.510
Co	0.01	1.440

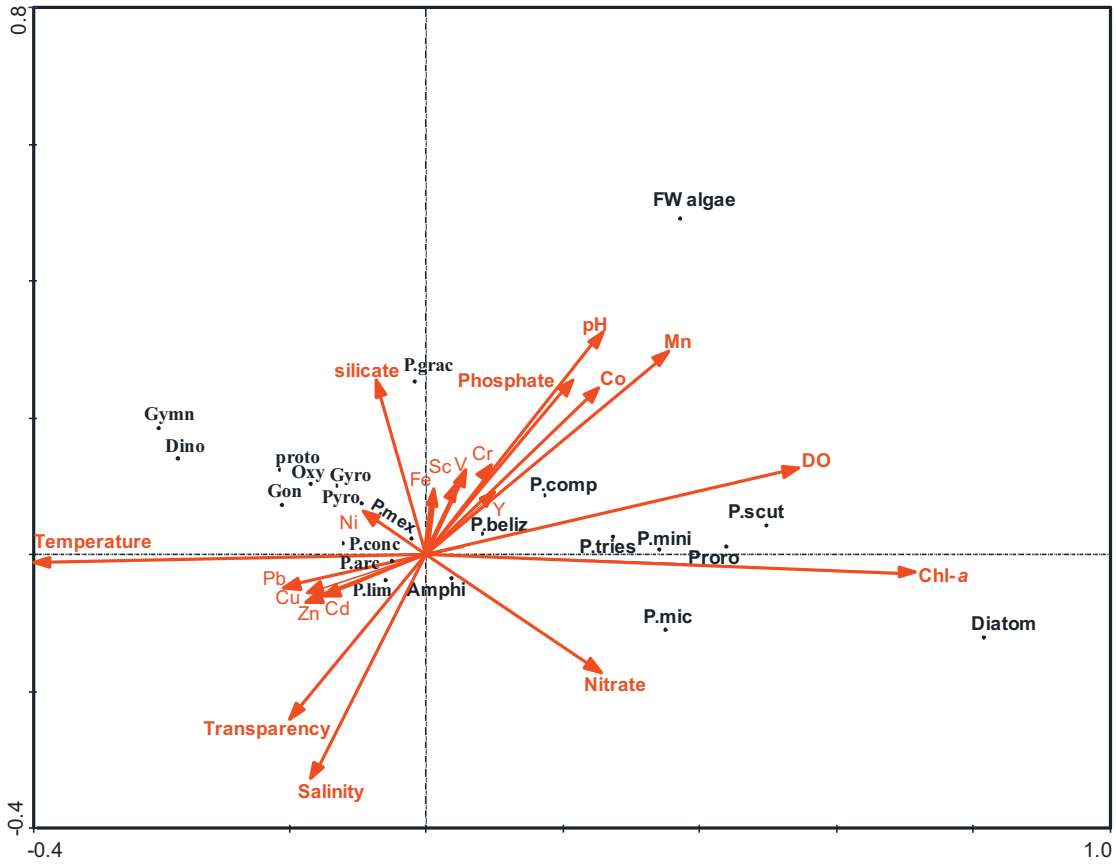

Figure 14. Results of RDA analysis illustrating phytoplankton species in relation to environmental variables. The significant variables are bold (See Fig.13 for abbreviations).

5. Discussion

The area of Abu-Qir Bay, particularly that found in the vicinity of discharged wastes is heavily polluted and referred as a hot spot (EEA, 2006). The high levels of nutrient salts and high concentration of chlorophyll-*a* indicate an acute eutrophication in the bay. In addition, the low concentrations of dissolved oxygen (reaches 0 mg/L in some stations) reflect the deterioration of water quality in the bay. These conditions altogether appeared to have pronounced impact on the qualitative and quantitative structure of phytoplankton especially dinoflagellate community in the bay.

It is well known from other related studies (Lohrenz et al., 2003; Nuccio et al., 2003; Song et al., 2004; Nowrouzi and Valavi, 2010), that some environmental parameters seem to play an important role in determining the phytoplankton community

abundance, favouring or limiting the growth of the different groups of phytoplankton. The RDA reveals that temperature is the most important factor that shapes phytoplankton community structure at all locations. The importance of temperature reflects the strong seasonal differences characteristic of temperate coastal waters. Furthermore, the productivity factor (Chl-*a*, nitrate, phosphate, and silicate) are also significantly linked to different phytoplankton community structures at different locations. Transparency, pH, and salinity appear also to be a significant factor shaping phytoplankton communities in the study area. Concerning the heavy metals, Mn and Co are the most important two metals affecting phytoplankton distribution.

The total phytoplankton counts are negatively correlated with temperature, while positively correlated with dissolved oxygen concentration and pH. This correlation explains the high outstanding peak of phytoplankton in winter especially at St.6. Penna et al. (2006) stated that dissolved oxygen and pH reached high values correspondence with phytoplankton bloom events. Other studies confirm the positive correlation between pH and phytoplankton production, where the phytoplankton photosynthetic activity is causing more consumption of carbon dioxide and the rise of pH (e.g. Abdalla et al., 1995; Labib, 1997; Zaghloul and Hussein, 2000; Mustapha, 2009). Radwan (2005) indicated that, the high phytoplankton productivity in winter was accompanied by the presence of high dissolved oxygen in winter due to the water movement by wind action and to the decrease in water temperature and increasing oxygen solubility, in contrast to the decreased dissolved oxygen concentration in summer due to low solubility of oxygen and increased rated of its utilization through biochemical reactions at high temperature.

During the present study blooms of dinoflagellates occur in summer (positive relationship with temperature), while in winter diatoms are dominant (negative relationship). This classical diatoms-dinoflagellate distribution was confirmed by several studies (Margalef, 1978; Smayda, 1980; Bologa, 1986; Benli, 1987; Caroppo et al., 1999; Mikhail 2001; Nassar and Hamed, 2003; Ismael, 2003; Eker-Develi, and Kideys, 2003; Gastiunaite et al., 2005). They indicated that the rise in temperature stimulate dinoflagellates productivity. More particularly, *Gymnodinium* spp. blooms in summer when favoured by high temperature. López – Flores et al. (2006)

indicated that *Gymnodinium* spp. is mainly detected during summer. Labib (1999) stated that chain forming dinoflagellates, *Gymnodinium* spp. achieved its massive red tide blooms during summer – early autumn.

In the present study the total dinoflagellates and their different species mainly *Gymnodinium* spp. proliferate in the warm saline stratified condition. In Abu-Qir Bay, surface water salinity decreases as a direct effect of inland water discharge, while the bottom water was less affected by sewage effluents and the water salinity remained high, so this stratification allows the growth of dinoflagellate. Several researchers confirmed this correlation (e.g. Ismael, 1998; Mikhail, 2001; Penna et al., 2006). They stated that, meanwhile the increased surface heating, and the pronounced salinity differences between the surface, and over the bottom created the suitable condition for the phyto - flagellates to bloom. Their occurrence appears to be most sensitive to growth inhibition by small-scale turbulence, sporadically appeared with a well developed thermo–haline stratified water column i.e. with increased stability of the water column. Labib (1999) stated that *Gymnodinium* spp. was dominated in water with low salinity and density stratified water column.

RDA reveals that the relation of freshwater algae with environmental parameters varies from positive with nutrient and negative with salinity. This is mainly due to the increase in nutrient is accompanied with the decrease of surface water salinity as a direct effect of inland water discharge. This usual negative correlation between Chlorophyceae, Cyanophyceae, and Euglenophyceae with salinity is related to the presence of freshwater species such as *Pediastrum* spp., *Merismopedia* spp., *Staurastrum* spp., *Euglena* spp., and *Spirulina* spp. The present findings are in agreement with Stirn (1988), Sarojini (1994) and Shams El-Din and Dorgham (2007) who observed that Chlorophyceae and Cyanophyceae are usually favoured by increased nutrients and dissolved organic material characterizing areas subjected to freshwater discharge.

The role of nutrient (phosphate and nitrate) in accelerating the existence of diatom production is expressed by its positive correlation with the nutrient except silicate and by its dominance in the highly eutrophicated stations such as St.6. Totti et al. (2000) reported that diatom distribution was known to be affected by continental, nutrient rich water input. The negative relation between diatoms and silicate is not

surprising since diatoms need silicate in order to build their frustules. Marasovic and Vukadin (1982) and Caroppo et al. (2006) reported that in the shallow ecosystems the effects of anthropogenic inputs of nutrients are more evident and phytoplankton standing crop, including diatom, is strongly related to such nutrients, mainly the N-compounds.

We record that the total dinophytes is positively related with silicate, while it is negatively related with nitrate and phosphate. This may indicates the minor role of nitrate and phosphate as controlling factor of dinoflagellate count in Abu-Qir Bay. This negative correlation with phosphate could be explained by that many of the common dinoflagellates may potentially be mixotrophic species, partly independent on ambient nutrient conditions (Jacobson and Andersen, 1994). For example López – Flores et al. (2006) reported that *Prorocentrum micans* is mixotrophy, while *Gymnodinium* spp., and *Amphidinium* spp. have all trophic strategy so they can adopt alternative nutritional strategies. Many dinoflagellate species also migrate vertically and are able to use subpycnocline nutrients during the stratified period, thus are independent of ambient nutrient conditions of the surface layer (Passow, 1991; Olli et al., 1998). Some dinoflagellates may also be limited by availability of trace metals that are bound in the humic substances (Graneli et al., 1985). This negative relationship could be also explained by the nutrient depletion during dinoflagellate bloom.

From RDA analysis we find that *Prorocentrum minimum* is related positively with nitrate, phosphate and negatively with salinity, temperature, and transparency. The results suggest that this potentially toxic species could well adapt to low salinity and temperature and occurred particularly in coastal waters, rich in nitrate and phosphate relative to silicate, this is because *P. minimum* is a eurythermal and euryhaline species (Grzebyk and Berland, 1996). This environmental condition enhancing the growth and proliferation of *P. minimum* in the bay coincide with those observed in the previous studies (e.g., Pertola et al., 2005). Several authors found that nutrient-rich coastal waters are common habitats for *P. minimum* around the world (Tyler and Seliger, 1978; Kimor et al., 1985; Silva, 1985; Sukhanova et al., 1988; Mendez, 1993; Hajdu et al., 2000; Smayda and Reynolds, 2001). There is previous suggestion that *P. minimum* attains a competitive advantage in relatively cool, turbid

environments influenced by runoff from land and rivers (Grzebyk and Berland, 1996). *Prorocentrum minimum* is also known as an invasive strategist, i.e., leading to success in somewhat eutrophied and stratified coastal waters (Smayda and Reynolds, 2001). It is worthy to note that this species is typical of enclosed and semi-enclosed basins as well as estuarine Mediterranean waters (Caroppo, 2000).

Regarding to the pollution aspect, which is the key point in this study, there are several biological indicators of pollution and eutrophication concluded from the present work.

Phytoplankton standing crop is a good estimate of the current degree of productivity. In the present study there is a pronounced increase in the total phytoplankton standing crop compared with previous study (Elsherif and Gharib, 1994; Ismael, 1998), which indicates the elevated eutrophication level in the Abu-Qir Bay through the last 20 years. Caroppo and Cardellicchio (1995) and Caroppo et al. (2006) reported that the higher phytoplankton abundances strengthen the general eutrophic nature especially of the semi-enclosed basin.

In the present study some dinoflagellates taxa have a heavy bloom especially *Gymnodinium* spp. (the most dominant taxon). Abdalla et al. (1995) reported that the blooming of some members of dinoflagellates may be considered as indication of eutrophication. Although dinoflagellates community is characterized by the presence a large number of taxa, few of them are responsible for the bulk of the population. This feature *per se* is a sign for pollution. Zaghloul and Hussein (2000) reported that the occurrence of algal blooms may indicate possible impacts of anthropogenic inputs on the ecosystem. Reduction in the number of dominant species, species diversity, and the increase in cell count of one or two algae are some of the changes observed in the phytoplankton populations of polluted environments. The low diversity in the study area is indicated by the low α-Fisher index values. The index values in most of the stations in the studied bay fall below the range of the values found by Murray (1973) for shelf seas ($\alpha > 5$), or just above the boundary. The exception is recorded in the offshore less eutrophicated, high diversified stations (e.g., St.8 and St.9).

The low dinoflagellate productivity at highly polluted stations such as St.4 and St.3 in spite of a relatively high nutrients level could be explained by the inhibiting effect of the industrial waste waters disposed into the bay from the Abu-Qir Fertilizer Company and El-Tabia pumping station. This can be confirmed by the inverse relation between dinoflagellate abundance and some heavy metals (Mn and Co).

During the study period, several toxic and harmful species (e.g. *Prorocentrum micans, Prorocentrum minimum, Prorocentrum lima, Dinophysis acuminata, Dinophysis caudata, Gymnodinium spp., Ceratium lineatum,* and *Amphidinium* spp.) are recorded in Abu-Qir Bay. The problem of marine eutrophication has been highlighted in recent years by the occurrence of increasingly severe toxic phytoplankton blooms in many near-shore waters worldwide. Toxic phytoplankton blooms in the sea are of even greater concern than in freshwater ecosystems (Smith et al., 1999).

In contrast to other dinoflagellate species, the present study indicates the ploriferation of *Prorocentrum* species mainly *Prorocentrum micans* and *Prorocentrum triestinum* in the highly polluted eutrophicated area. Therefore we could consider these species as pollution resistant or indicator. *Prorocentrum micans* is a neritic euryhaline form, well known to the Egyptian Mediterranean waters (Gergis, 1983; Dowidar et al., 1983; Dorgham and Osman, 1987; Samaan and Mickhail 1990; ElSherif and Gharib 1994; Ismael, 1993; Ismael and Dorgham, 2003; Gharib and Dorgham, 2006) and it is considered as indicator of eutrophication and pollution (Pagon, 1985; Dorgham et al., 1987; Zaghloul, 1994). Zaghloul and Halim (1990), and Zaghloul (1994) considered *Prorocentrum triestinum* as indicator of eutrophication. *Prorocentrum triestinum* is a well known red tide species in temperate coastal waters, its occurrence is closely related to land drainage (Lizuka, 1985; Labib, 1995).

Zaghloul (1994) classified *Ceratium* spp. as a sensitive species to pollution. The sensitive species are often express that sensitivity through their absence in polluted habitats. Consequently, conclusions about the pollution situation in an area should not be drawn by noting the absence of certain species, unless it can be documented that they actually have disappeared from the area (Alve, 1995). In the present study *Ceratium* taxon has a very small percentage composition and only few species (4) are

recorded. In contrast, Ismael (1998) found that *Ceratium* spp. represented 20.5% of the total community with 21 species and varieties in Abu-Qir bay. Thus, this work confirms that the paired use of both pollution tolerant and sensitive indicator species is the best option for monitoring pollution. Alvarez Cobelas et al. (1994) and Dokulil and Padisak (1994) reported shifts in species composition related to eutrophication. Over the last few decades, Mediterranean marine biodiversity has been undergoing rapid alterations due to climate change and human impact (Turley, 1999). On the other hand, the freshwater species *Ceratium hirundinella* is regarded as indicators of meso-eutrophic in lake within the city of Poznań (western Poland) (Wasielewska, 2006), but this species has no records in our study area. It is worthy to note that the temporal distribution of *Ceratium* species over the last century showed a progressive disappearance from the surface layer of likely stenothermic species, which may have moved to deeper layers in response to water warming, along with a decrease of species richness during the annual cycle. A change in the overall species assemblage also occurred from past to present in the Ligurian Sea, suggesting a warming in this area consistent with the development in surface water temperatures (Tunin-Ley et al., 2009). They suggested that *Ceratium* species may constitute good biological indicators of warming in the NW Mediterranean Sea.

The zooplankton cycle has the same seasonal pattern as phytoplankton. The maximum and minimum abundances are recorded in February and May respectively. This unusual positive relation reflects eutrophic state in Abu Qir Bay during the period of this work. Such situation provides a warning alarm against the increased pollution in the Bay. Zaghloul and Hussein (2000) reported positive correlation coefficient between phytoplankton and zooplankton standing crop in Lake Edku which was an indicator of eutrophication.

6. Conclusion

As conclusion, it can be stated that Abu-Qir Bay is highly eutrophicated and polluted with excessive nutrient and heavy metal loading from surrounding areas. Such environmental condition of the bay might also alter the structure and function of the plankton community with excessive increase in primary production, frequent occurrence of toxic dinoflagellate, but with reduced diversity. In general, the bay sustained a high phytoplankton standing crop with higher diversity relatively towards

the outer than the interior zone in terms of Fisher's alpha (α) diversity index. Both of environmental parameters and anthropogenic effects seem to play an important role in determining the phytoplankton community abundance, composition, and then diversity, favouring or limiting the growth of the different groups of the phytoplankton. Multivariate analyses indicate that primary production of the bay is limited by temperature, nutrient concentrations, dissolved oxygen, heavy metals concentration, transparency, pH, and salinity. Dinoflagellate bloom is recorded in summer, while diatoms are dominated in winter. The summer bloom of *Gymnodinium* spp. is related to warm saline stratified condition in the bay. The structure of both diatom- and fresh-water algae dominated communities is governed by nutrient and salinity. Heavy metal pollution has a severe negative effect on the dinoflagellate abundance, which is reflected on its low abundance in the polluted stations. *Prorocentrum* species, mainly *P. micans* and *P. triestinum*, are favoured by high eutrophication-pollution level. Therefore this species could be used as valuable indicator for eutrophicated polluted area, while *Ceratium* spp. could be considered as sensitive species

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Appendix 1. Seasonal and spatial variations of temperature (°C), surface and bottom salinity (‰), pH, transparency (m), DO (mg/L), Chl-*a* (µg/L), nitrate, phosphate, and silicate concentrations (µg at/L) in Abu-Qir bay. Abbreviations: T: temperature, S: surface salinity, BS: bottom salinity, Tra: transparency, N: nitrate concentration, Si: silicate concentration, P: phosphate concentration, Aug: August, Nov: November, and Feb: February.

Stations	T May	T Aug	T Nov	T Feb	Regional average	S May	S Aug	S Nov	S Feb	Regional average
St.1	25.00	30.00	21.50	19.50	24.00	39.30	38.01	37.52	31.00	36.46
St.2	25.00	30.00	25.00	19.50	24.88	39.20	37.76	-	29.80	35.59
St.3	26.00	30.00	22.50	20.00	24.63	3.70	20.28	36.26	9.30	17.39
St.4	26.00	32.50	25.00	19.50	25.75	9.40	15.24	34.49	18.00	19.28
St.5	24.80	30.00	22.00	18.00	23.70	26.30	25.50	38.81	33.40	31.00
St.6	24.50	30.00	21.50	18.00	23.50	14.00	17.99	5.72	16.40	13.53
St.7	24.80	30.00	22.50	18.00	23.83	39.60	35.59	36.19	37.90	37.32
St.8	22.80	29.90	23.00	19.00	23.68	39.80	36.74	37.41	37.20	37.79
St.9	24.00	30.00	24.50	18.00	24.13	38.10	37.41	28.60	33.80	34.48
Seasonal average	24.77	30.27	23.06	18.83	24.23	27.71	29.39	31.88	27.42	29.20
Stations	BS May	BS Aug	BS Nov	BS Feb	Regional average	pH May	pH Aug	pH Nov	pH Feb	Regional average
St.1	39.50	37.99	38.93	37.80	38.55	8.16	8.29	8.22	8.00	8.17
St.2	39.20	-	-	-	39.20	8.20	8.19	7.71	7.85	7.99
St.3	-	-	36.26	-	36.26	7.30	7.33	7.27	7.50	7.35
St.4	32.00	27.85	38.21	30.00	32.01	7.44	7.65	8.34	7.45	7.72
St.5	37.90	35.28	36.83	37.30	36.83	8.17	8.54	8.13	8.10	8.24
St.6	38.70	36.26	38.89	36.90	37.69	8.50	8.40	9.04	8.60	8.64
St.7	39.70	38.29	37.34	38.60	38.48	8.17	8.43	8.16	8.15	8.23
St.8	39.80	38.29	39.04	38.60	38.93	8.10	8.37	8.32	8.05	8.21
St.9	39.70	37.82	38.41	37.70	38.41	8.22	8.25	7.63	7.95	8.01
Seasonal average	38.31	35.97	37.99	36.70	37.37	8.03	8.16	8.09	7.96	8.06
Stations	Tra May	Tra Aug	Tra Nov	Tra Feb	Regional average	DO May	DO Aug	DO Nov	DO Feb	Regional average
St.1	0.80	1.50	0.75	0.50	0.89	4.60	2.72	3.20	4.34	3.72
St.2	0.10	0.05	0.30	0.40	0.44	2.00	1.72	1.60	1.15	1.62
St.3	0.20	0.05	0.10	0.00	0.09	0.00	0.00	0.00	1.02	0.26
St.4	0.30	0.02	0.30	0.05	0.17	0.00	0.07	1.28	0.94	0.57
St.5	0.50	0.30	0.75	0.30	0.46	5.30	4.83	1.92	3.66	3.93
St.6	0.60	0.30	0.30	0.30	0.38	2.72	4.76	4.00	7.99	4.87
St.7	3.00	1.00	1.25	1.25	1.63	1.99	3.13	2.24	4.08	2.86
St.8	3.00	1.40	1.00	0.75	1.54	3.90	3.40	4.08	4.42	3.95
St.9	0.70	0.80	0.90	0.30	0.68	4.90	1.90	0.96	3.74	2.88
Seasonal average	1.12	0.60	0.63	0.43	0.70	2.82	2.50	2.14	3.48	2.74
Stations	Chl May	Chl Aug	Chl Nov	Chl Feb	Regional average	N May	N Aug	N Nov	N Feb	Regional average
St.1	15.34	4.20	3.19	10.93	8.42	13.68	4.00	23.76	6.67	12.03
St.2	23.25	21.75	5.21	13.55	15.94	4.25	1.24	-	-	2.74
St.3	7.34	9.71	1.58	12.01	7.66	6.72	20.31	-	9.83	12.29
St.4	11.77	6.05	6.99	1.28	6.52	3.07	3.69	50.00	53.38	27.53
St.5	18.55	28.80	8.73	16.51	18.15	-	1.52	32.08	7.05	13.55
St.6	19.52	45.25	10.63	48.25	30.91	21.68	5.18	-	33.42	20.09
St.7	6.11	0.10	4.41	0.38	3.63	1.19	2.82	12.77	12.39	7.29
St.8	1.65	0.01	0.58	1.09	0.83	1.25	1.40	1.60	1.07	1.33
St.9	7.18	0.40	2.68	13.31	5.89	1.45	4.34	10.00	8.33	6.03
Seasonal average	12.30	14.52	4.89	13.03	10.88	6.66	4.94	21.70	16.52	11.43
Stations	Si May	Si Aug	Si Nov	Si Feb	Regional average	P May	P Aug	P Nov	P Feb	Regional average
St.1	45.00	25.00	12.00	31.00	28.25	14.33	0.81	1.43	3.94	5.13
St.2	24.00	10.00	61.00	27.00	30.50	6.81	0.99	3.85	4.75	4.10
St.3	115.00	237.00	6.00	199.00	139.25	11.19	9.94	0.90	13.61	8.91
St.4	64.00	217.00	22.00	151.00	113.50	14.96	11.28	1.61	11.55	9.85
St.5	14.00	37.00	10.00	10.00	17.75	5.28	1.16	1.97	4.92	3.33
St.6	44.00	112.00	61.00	28.00	61.25	0.54	4.39	17.46	16.84	9.81
St.7	14.00	7.00	6.00	36.00	15.75	2.24	0.54	0.63	1.88	1.32
St.8	17.00	9.00	16.00	37.00	19.75	1.34	1.08	0.63	5.37	2.10
St.9	13.00	21.00	97.00	1.00	33.00	3.49	0.54	5.37	3.58	3.25
Seasonal average	38.89	75.00	32.33	57.78	50.47	6.69	3.41	3.76	7.38	5.31

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